WATER QUALITY'S INFLUENCE ON THE OCCUPANCY OF TWO JEOPARDIZED FISHES: THE BLACKSIDE DACE (*CHROSOMUS CUMBERLANDENSIS*) AND THE CUMBERLAND ARROW DARTER (*ETHEOSTOMA SAGITTA*) IN NORTHEAST TENNESSEE

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Master of Science

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by

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Accepted by the faculty of the College of Science, Morehead State University, in partial fulfillment of the requirements for the Master of Science degree.

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WATER QUALITY'S INFLUENCE ON THE OCCUPANCY OF TWO JEOPARDIZED FISHES: THE BLACKSIDE DACE (*CHROSOMUS CUMBERLANDENSIS*) AND THE CUMBERLAND ARROW DARTER (*ETHEOSTOMA SAGITTA*) IN NORTHEAST TENNESSEE

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The Cumberland River drainage in northeastern Tennessee hosts several rare endemic fish species, including the Blackside Dace (*Chrosomus cumberlandensis*) and the Cumberland Arrow Darter (*Etheostoma sagitta*). Declines of these two species have been attributed to anthropogenic disturbances (primarily logging and coal extraction). This research examined abundance and distribution of these two species at 47 sites within small streams located in Scott, Claiborne, and Campbell counties in Tennessee. Backpack electrofishing was employed to sample fishes in a quantitative protocol designed to allow analysis of fish distribution and abundance, using occupancy modeling analysis. To determine how water quality affects the distribution and abundance of these target species temperature, dissolved oxygen (DO), conductivity, pH, total suspended solids (TSS), alkalinity, dissolved NH_3, dissolved NO_3^-, dissolved SO_4^{2-}, dissolved Fe, dissolved PO_4^{3-}, total nitrogen (TN), and total phosphorus (TP) were measured. Blackside Dace were only encountered at ten sites; while Cumberland Arrow

Darters were observed at 18 sites. Pair-wise comparisons indicated TN and Kentucky Index of Biotic Integrity (KIBI) were significantly different ($p<0.050$) between sites with and without Blackside Dace. Conductivity and KIBI were statistically different ($p<0.050$) between sites with and without Cumberland Arrow Darters. Principle component analysis (PCA) indicated occupied sites had higher concentration of NO_3^-, and lower pH, conductivity, alkalinity, SO_4^{2-}, Fe, and NH_3 when compared to unoccupied sites for both species. Occupancy of Blackside Dace in this study was estimated to be 0.1531, and Cumberland Arrow Darter occupancy to be 0.3869. Using conductivity as a covariate, occupancy of Blackside Dace was higher in streams below 343 μS/cm (0.1934) than in streams with conductivities above 343 μS/cm (0.0749). Cumberland Arrow Darters showed the same trend; sites with conductivities above 343 μS/cm (0.1409) had lower estimated occupancies than sites with conductivities below 343 μS/cm (0.5089). This study supports the findings of previous research indicating these fishes respond to changes in water quality, and that water quality measures, especially conductivity, can serve as a tool to monitor stream disturbances that affect fishes. This study provides independent support for the use of conductivity as a monitoring tool, and its strong association with these rare fishes' distribution and abundance.

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Chapter 1: Introduction

The southeastern United States is an area of high biodiversity, especially within the plentiful aquatic habitats (Burr and Mayden 1992, Warren et al. 2000). The region has over 660 species of native fishes, but due to small population sizes, habitat fragmentation, and anthropogenic stress, this region also has the highest number of imperiled fishes in North America (Warren et al. 2000, Jenkins et al. 2015). The Cumberland River drainage above Cumberland Falls in southeastern Kentucky and northeastern Tennessee is home to several species of rare and endemic fishes. Two of these are *Chrosomus cumberlandensis* (Starnes and Starnes 1978) (Blackside Dace) and *Etheostoma sagitta* (Jordan and Swain 1883) (Cumberland Arrow Darter). Blackside Dace are listed federally as "threatened" (Biggins 1987) and as "endangered" by the International Union for Conservation of Nature (IUCN) (NatureServe 2014a). They also are listed as "threatened" in both Kentucky and Tennessee (Tennessee Wildlife Commission 2000, Kentucky Department of Fish and Wildlife Resources 2017, KSNPC 2010). Cumberland Arrow Darters are listed as "near threatened" by the IUCN (NatureServe 2014b), "imperiled" by the state of Tennessee (Withers 2012), and as a species of "special concern" in Kentucky (KSNPC 2010). The Appalachian region inhabited by these two species is rich in natural resources such as coal, natural gas, and timber. Historic and current extraction of these resources has resulted in changes to the abiotic and biotic environment, which results in degradation of aquatic communities (Olem 1988, KDOW 2000, Koel and Peterka 2003, Allen 2004).

Anthropogenic changes to stream habitats within this region has led to changes in aquatic biota composition and community dynamics (Etnier and Starnes 1993, Pond 2004, Hartman et al. 2005). Disturbance events that remove forest canopy cover can cause elevated water temperatures by increasing direct sunlight exposure. Land disturbances can create drastic changes in stream nutrient and ion loadings that result in changes to the benthic macroinvertebrate community. For example an allochthonous stream that no longer receives leaf pack because of deforestation will lose the benthic shredder insect community (Wetzel 2001). Changes in flow regime because of damming (by either beavers or humans) or watershed changes via paving, urban development, and/or logging also result in changes to aquatic communities. Damming can change habitats from lotic to lentic leading to reductions and replacements in the biotic community because of niche changes. Urbanization and other land disturbance events can lead to increased flash flooding via sheet flow from impervious surfaces (e.g. roads, roofs, concrete, exposed rock, etc.) (Wetzel 2001). Deforestation results in the loss of vegetation that absorbs and buffers streams from high water events and collects and stores nutrients.

Increasing water temperatures results in higher metabolic demands for heterothermic exotherms, such as fish. The oxygen-and-capacity limitation of the thermal tolerance hypothesis explains that two critical temperatures exist for heterotherms; at these points the maximal metabolic rate is equal to the standard metabolic rate (Moyes and Schulte 2016). When these two points are equal the animal's metabolic rate is only sufficient to maintain life, not any other activities such as locomotion. This temperature can result in death if experienced for extended periods. Changes to an ecosystem that alters the temperature for extended periods can result in the loss of species already living close to a critical thermal point. Changes in ion loadings, total suspended solids (TSS), salinity, and dissolved nutrients can lead to osmotic stress for many aquatic species.

Blackside Dace Biology

Taxonomic revision by Strange and Mayden in 2009 moved Blackside Dace from the original genus of *Phoxinus*, as described by Starnes and Starnes (1978), to the genus *Chrosomus* (Figure 1). Blackside Dace are in the family Cyprinidae (minnows) from the order Cypriniformes in the class Actinopterygii. Blackside Dace are one of 10 endemic fish species found in the Cumberland River drainage (Figure 2).

Many studies have been conducted that describe their life history, reproductive biology, habitat requirements, and ecology (Starnes and Starnes 1981, O'Bara 1988, Cicerello and Laudermilk 1996, Mattingly and Black 2013, Hayes 2015). Starnes and Starnes (1981) described Blackside Dace preferred habitat as Appalachian streams with cool, clear, and clean water surrounded by mature forests dominated by hemlock (*Tsuga canadensis* and *caroliniana*) and *Rhododendron* sp. Streams occupied by Blackside Dace typically have undercut banks with cover such as stumps, root masses, or other dense vegetation (Baxter 1997, O'Bara 1988, Starnes and Starnes 1981).

Hitt et al. (2016) incorporated 16 variables (including geophysical and hydrological characters, land use, and water quality) into boosted regression trees to determine what factors best predicted Blackside Dace presence. The study compiled 249 Kentucky samples from 2003 to 2012. Conductivity had a relative importance of 15.86%, while temperature, stream slope, and watershed area each had at least 10% relative importance in predicting Blackside Dace occurrence. Hitt et al. (2016) estimated the threshold for response to conductivity for Blackside Dace to be 343 µS/cm (95% CI: 123–563 µS/cm). Prior to this a study by Black et al. (2013) looked at factors influencing the presence of Blackside Dace within both Kentucky and Tennessee. Black et al. (2013) found that Blackside Dace are more likely to be present when the conductivity is <240 µS/cm. Hitt et al.'s (2016) prediction for response falls within 50 µS/cm of the USEPA (2011) conductivity

Figure 1. Image of a Blackside Dace encountered at Rose Creek on 3-August 2015.

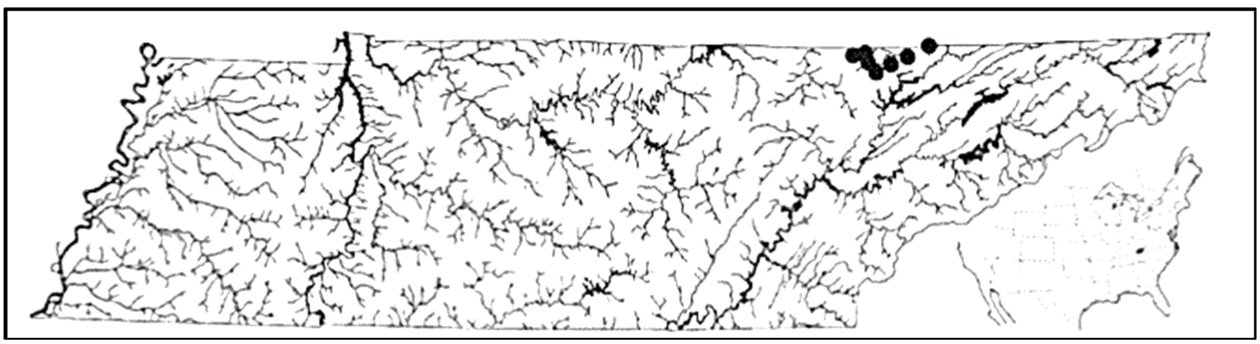

Figure 2. Distribution of Blackside Dace in Tennessee (Etnier and Starnes 1993).

benchmark of 300 µS/cm, but is higher than Black et al.'s (2013) estimation of 240 µS/cm. Hitt et al. (2016) used a different method, and a larger and more recent dataset than Black et al. (2013) to estimate the conductivity change point. Jones (2005) in a predictive model suggested that Blackside Dace are more persistent in streams with conductivities below 240 µS/cm. Hitt et al. (2016) explains that the results are consistent with, and not contradictory to, previous studies, because these studies support conductivity and stream temperature as being the primary predictors of Blackside Dace occurrence. While the aforementioned studies provide informative results, they must be weighted appropriately. These studies compiled data collected from multiple years by multiple researchers, and in some cases only from one state rather than the entire range of Blackside Dace. Many studies, like the ones mentioned, could be criticized because they "cherry picked" sites based upon presumed quality and/or ease of access, possibly introducing bias in the analysis.

Blackside Dace streams have been reported to have summer temperatures between 14.6 and 18.5°C by both Black et al. (2013) and Jones (2005). Roghair and Whalen (2001) found that streams containing Blackside Dace did not exceed 23°C during their study where they monitored water temperatures at dawn, mid-day, and dusk. Black et al. (2013) reported that streams observed to contain Blackside Dace had dissolved oxygen (DO) levels above 8.5 mg/L, and turbidity less than 10 Nephelometric Turbidity Units (NTU). Roghair and Whalen (2001) also found that streams with Blackside Dace did not have a gradient higher than 4%.

Blackside Dace are rarely a common species and may often only be found only in a few pools within a 1 km reach (Starnes and Starnes 1981). Starnes and Starnes (1981) noted that *Pimephales notatus* (Rafinesque) (Bluntnose Minnow), *Semotilus atromaculatus* (Mitchill) (Creek Chub), *Catostomus commersonii* (Lacepede) (White Sucker), *Etheostoma kennicotti*

(Putnam) (Stripetail Darter), and Cumberland Arrow Darters were typically collected with Blackside Dace. Raney (1947) documented *Campostoma anomalum* (Rafinesque) (Central Stoneroller) also commonly occurring with Blackside Dace. Starnes and Starnes (1981) explained that the Bluntnose Minnow could be a competitor for food with Blackside Dace, while the reported diets of other re-occurring species differed from Blackside Dace.

Blackside Dace diets vary throughout the year based upon the available food sources. Digestive tract research by Starnes and Starnes (1981) found the main component of the gut to be sand, often ingested with the food source. Starnes and Starnes (1981) suggest that sand could be intentionally ingested to serve as a type of gastrolith to aid in breaking down plant material, or it could be a byproduct of consuming detritus. Starnes and Starnes (1981) state the passage of sand causes diatom and algal cells to rupture, thus releasing nutrients to the digestive tract. The second most abundant component of the gut was unidentified organic material, such as root hairs, decaying plant or animal material, or leaf fragments. Algae and diatoms also were found to be part of the Blackside Dace's diet. Common genera of algae were *Oedegonium* and *Spirogyra*, while common genera of diatoms were *Cymbella*, *Navicula*, and *Lyngbya* (Starnes and Starnes 1981). Macroinvertebrates such as Chironomidae and Hydropsychidae also contribute to the diet, especially in the winter (Etnier and Starnes 1993, Starnes and Starnes 1981).

Mattingly and Black (2013) reported Blackside Dace sharing nests with Creek Chubs, where they argue that Blackside Dace spawning is limited by nest building cyprinids. They also noted that the nest cleaning actions of the host cyprinid provided a clean gravel substrate suitable for spawning. Etnier and Starnes (1993) reported that Blackside Dace use clean gravel riffles when Central Stonerollers or Creek Chub nests are not available. Cicerello and Laudermilk (1996) observed Blackside Dace spawning in Creek Chub nests in Rock Creek in McCreary

County, Kentucky. The population observed by Cicerello and Laudermilk (1996) had 30+ individuals spawning in the same nest at the downstream end of a pool, just above a small riffle.

Conservation efforts by the USFWS for Blackside Dace were outlined in the 1988 recovery plan that focused on the preservation of existing populations, finding unknown populations, and protection of habitat (USFWS 1988). Blackside Dace are known from 205 locations along 113 streams, based upon all known historic and current data compiled by the Kentucky State Nature Preserve Commission (KSNPC 2010). Starnes (1981) estimates Blackside Dace have been extirpated from 60-70% of their historic range, and most extant populations are reduced, and persist as remnant populations (Black and Mattingly 2007).

Cumberland Arrow Darter Biology

Cumberland Arrow Darters belongs to the family Percidae from the order Perciformes of the class Actinopterygii and are endemic to the Cumberland River Drainage (Figures 3 and 4). The Kentucky River drainage contains a very similar species that is now recognized by USFWS (Floyd 2014) as the Kentucky Arrow Darter *(Etheostoma spilotum*). The Kentucky River and Cumberland River populations previously were regarded as subspecies of a single species (Burr and Warren 1986, Etnier and Starnes 1993). The Kentucky Arrow Darter population has greatly declined, resulting in a threatened listing by the USFWS (USFWS 2016). The Kentucky Arrow Darter will receive protection from USFWS, but to date no recovery plan or critical habitat has been listed for the species.

Hitt et al. (2016) found the conductivity abundance change point to be 261 µS/cm (with a 95% confidence interval of 151–370 µS/cm) for the Kentucky Arrow Darter. Cumberland Arrow Darters can typically be found in slow to moderately flowing streams characterized by rocky substrates with sandy patches and small gravel areas (Etnier and Starnes 1993, Lowe 1979). Streams containing Cumberland Arrow Darters often are surrounded by dense riparian zones comprised of hemlock, rhododendron, and deciduous hardwoods (Etnier and Starnes 1993). Stream waters usually do not exceed 21°C (Etnier and Starnes 1993), but Lowe (1979) reports collecting Cumberland Arrow Darters in stream temperatures as warm as 37°C during the summer of 1975 in the Stinking Creek system, Anderson County, Tennessee. Lowe (1979) also reports the species appeared to be somewhat silt-tolerant, as these darters occurred in the Hickory Creek system, where siltation was moderate and consistent during their study.

Cumberland Arrow Darters reach sexual maturity within the first year and begin spawning the following year (Lowe 1979). Lowe reported that spawning activities occurred under rocks of moderate size (15-18 cm observed) where the substrate is sand and small gravel 6-12 mm in diameter. The males create nests under stones suitable for mating in an area of the stream where they will court females. Spawning occurs in cold waters during the spring, typically in April (Etnier and Starnes 1993). Etnier and Starnes (1993) suggest that the males likely protect the nest until the eggs hatch.

Cumberland Arrow Darters are almost exclusively insectivorous as adults (Lowe 1979, Starnes and Starnes 1981), but as juveniles they prey mainly on copepods, cladocerans, and larval dipterans (Lowe 1979). Lowe (1979) found that Cumberland Arrow Darters do not feed on drifting food but rather consume live benthic invertebrates. The main constituents of the adult diet were Ephemeroptera (Baetidae sp., Heptageniidae sp., and Ephemeridae sp.) and immature Dipterans (Simuliidae sp.) (Lowe 1979). Lowe (1979) found that as adults increased in size so did the size of the prey consumed. Lowe also found decapods, Coleoptera, Plecoptera immatures, and other fish were being consumed by 3-4 year old Cumberland Arrow Darters. The difference in the diets and spawning activities of Blackside Dace and Cumberland Arrow Darters allows them to peacefully coexist.

Figure 3. Image of a Cumberland Arrow Darter encountered at an unnamed tributary of Laurel Creek on 11-August 2015.

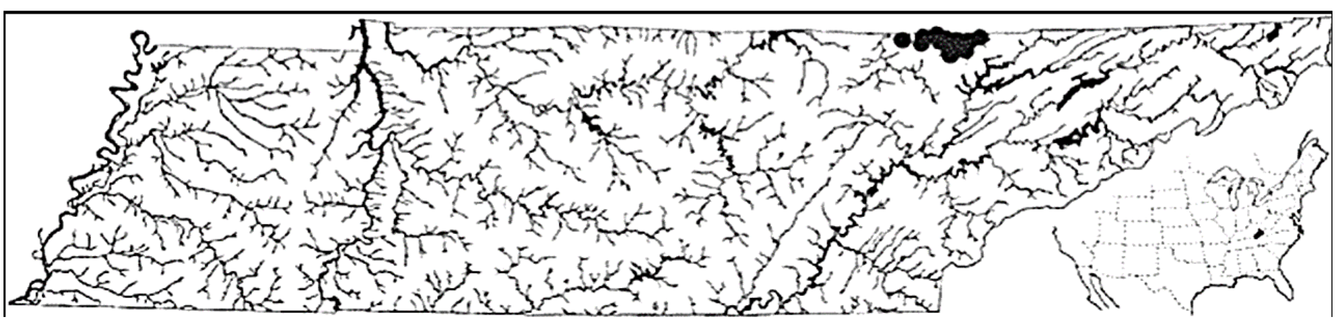

Figure 4. Distribution of Cumberland Arrow Darter within Tennessee (Etnier and Starnes 1993).

Regional Threats to Aquatic Fauna

Extensive surface mining in the region is harmful to both species, however, Etnier and Starnes (1993) and Lowe (1979) suggest that the Cumberland Arrow Darter is more tolerant than Blackside Dace to habitat changes from land disturbances. Within the region, stream ecosystems also are being degraded by contaminated runoff, and destruction of the riparian zones via logging, road development, and agricultural practices (Starnes and Starnes 1978, O'Bara 1990). Land disturbances in the region have not only led to habitat loss for fishes, but also for other aquatic fauna, their macroinvertebrate food supply (Bradfield 1986, Green et al. 2000, Chambers and Messinger 2001, Pond 2010, USEPA 2011). Koel and Perterka (2003) found that loss of aquatic integrity consequently leads to changes in the fish assemblages, and increased land use influences streams at an exponential rate. Bernhardt and Palmer (2011) reported that as disturbance increases within a watershed impacts to streams and aquatic biota increase.

Coal mining and logging are the two main threats to Blackside Dace and Cumberland Arrow Darters, but other practices, and even invasive species establishment, are causing changes within the region. Row crops, untreated sewage, roads, and the Hemlock Wooly Adelgid (*Adelges tsugae*) are affecting stream communities in the Cumberland River drainage (Starnes and Starnes 1978, O'Bara 1990, Paul and Meyer 2001, Bloomquist et al. 2010). Nutrient loading from row crops and untreated sewage discharged directly into the streams are increasing nutrient conditions that can lead to algae blooms, resulting in lowered DO (Mattingly et al. 2005). The creation of roads within this region requires many bridges, typically as culverts, that can serve as barriers to fish movements (Eisenhour and Floyd 2013). Corsi et al. (2010) point out that salting roads during the winter months leads to elevated ions and conductivities within streams. Extirpation of hemlocks by deforestation or pathogens, will lead to increased water temperatures as canopy disappears, leading to increased solar warming (Bloomquist et al. 2010). Bloomquist et al. (2010) also claims that loss of hemlocks will increase sedimentation rates because of reduced root mass to hold soil in place and increase the intensity of stream flow fluctuations during rain events because of increased runoff.

The most prevalent disturbance in the region, coal mining, has been known to negatively affect streams by lowering pH (Brake et al. 2001) and increasing conductivity (Brake et al. 2001), iron (Olem 1988), sulfur (Lindberg et al. 2011), and siltation (Minear and Tschantz 1976). Bernhardt and Palmer (2011) found that when coal mines exceed 5.4% of the watershed area the biotic integrity of streams begins to decline. Arnwine et al. (2014) found that of the 1,003 stream miles impaired by coal mining within Tennessee, 87% of impairment is caused by abandoned mines, where the leading causes of harm were low pH, and high iron, manganese, and silt deposition on streambeds. In streams influenced by coal mining, Minear and Tschantz (1976) found iron concentrations in excess of 1.5 mg/L and sulfate concentrations above 1000 mg/L.

Lindburg et al. (2011) sampled 72 tributaries in the upper Mudd River drainage, West Virginia and found that streams unaffected by coal mining had an average conductivity of 156.1±11.6 µS/cm, whereas coal influenced streams had an average conductivity of 1293.9±11.6 µS/cm. Cormier et al. (2011) found that conductivities above 300 µS/cm were associated with declines to freshwater aquatic life. This became the Environmental Protection Agency standard for conductivities in the Appalachian region (USEPA 2011). Bernhardt and Palmer (2011) conducted a study in West Virginia where reference streams had a mean conductivity of 64 µS/cm, while unmined streams had an average of 118 µS/cm, and mined streams averaged 626 µS/cm. Bernhardt and Palmer (2011) also provide that conductivities > 308 µS/cm would cause biological impairment. High conductivity can stress aquatic life by requiring more energy to maintain osmotic balance (Koel and Peterka 2003, Oliva-Paterna et al. 2003). Conductivity could be serving as an indicator for some other adverse water quality factor influencing the aquatic community.

Minear and Tschantz (1976) found streams unaffected by coal mining in the New River watershed, West Virginia, had total nitrogen (TN) and total suspended solids (TSS) values below 50 mg/L while affected streams were regularly above 100 mg/L. Conductivity can increase with TSS levels, but this is not always the case. Eight major ions [K^+, Na^+, Ca^{2+}, Mg^{2+}, SO_4^{2-}, Cl^-, HCO_3^-, and CO_3^{2-}] contribute to the conductivity of freshwaters in North America (Wetzel 2001). Conductivity is measured as the reciprocal of the resistance of a solution to the flow of electricity from two electrodes each with a surface area of 1 cm^2 that are 1 cm apart. Material suspended in the water (measured by TSS) may or may not be ionic, thus conductivity is not always linked to TSS but can be influenced by it.

Occupancy Modeling

Occupancy modeling was developed in the early 2000s to solve the unavoidable problem of imperfect detection when studying species distributions and to investigate what factors are determining their distribution, or to study metapopulations (MacKenzie et al. 2006). Occupancy models account for imperfect detection by using presence/absence data from multiple visits to the same site. When a species is not found in a field survey, it could be due to true absence, or it could have been present, but not detected (a false negative). To solve this problem MacKenzie et al. (2002, 2003, and 2004) designed the occupancy models used by USGS Presence software. This estimates the probability of detection (and false negatives) by resampling. The assumptions of occupancy models are: the occupancy state is closed, sites are independent, there is no unexplained heterogeneity in occupancy, and detectability at each occupied site is the same (USGS 2005).

When sampling fishes it is difficult, logistically, to resample multiple streams. Also, repeat sampling, especially involving electrofishing, can overly stress sensitive fish species. The repeated sampling problem is typically solved, in fish applications, by having multiple, independent sampling plots at each site (Albanese et al. 2007, Kendall and White 2009). Sampling this way does not break the assumptions laid out for the occupancy models, and solves the cost and logistic problems. Occupancy modeling was used to help understand the relationships between fishes and water quality factors.

Study Goals & Objective

Many previous studies, described above, have examined Blackside Dace and Cumberland Arrow Darters ecology by assessing habitat, water quality, geomorphology, hydrology, and the association of land use. Many studies have also only focused on the Kentucky portion of Blackside Dace habitat, and not incorporated the Tennessee portion. This study was designed to remove bias to produce results that best represent the entire region and the fishes therein. The objective was to use measure and analyze water quality variables to determine if there is a connection between the physiochemical characteristics of the water itself and these rare fish's occurrence. This study examines 13 water quality factors on a continuous scale, rather than simply categorizing them as "low", "moderate", or "high". Water quality factors such as conductivity, temperature, DO, pH, alkalinity, dissolved SO_4^{2-}, Fe, NO_3^-, NH_3, TSS, total nitrogen (TN), and total phosphorus (TP) are assessed at 47 randomly-selected sites in the upper Cumberland River drainage within Scott, Campbell, and Claiborne counties in northeastern Tennessee. The first objective of this study was to estimate the occupancy of Blackside Dace and Cumberland Arrow Darters, and ,seven other commonly-occurring fishes within the region. The second objective – was to determine the relationship between diversity, fish community health, and target species presence. The third objective was to assess relationships between 13 water quality parameters and target species' presence. The final objective was to assess the relationship between conductivity and occupancy. Conservation efforts for Blackside Dace and Cumberland Arrow Darters could be aided by the objective nature of this study and the potential independent support for the use of conductivity as an indicator of stream health.

Chapter 2: Methods

Site Selection

Forty-seven sites from 37 streams in the upper Cumberland River Drainage from Scott, Claiborne, and Campbell counties in northeast Tennessee were selected via a stratified random, using ArcGIS software following the randomization procedures used by the USEPA (2006) and Olsen and Peck (2008) (Figure 5). Streams with watersheds greater than 1.30 km^2 were chosen because they were most likely to support Blackside Dace or Cumberland Arrow Darters. The number of sites to be sampled was determined by using a method similar one used by MacKenzie et al. (2005) that a standard error of 0.05 for occupancy modeling. This method provides an effective cost/benefit approach to maximize results for efforts expended. This method suggested a minimum of 42 streams be sampled to have achieve a standard error of 0.05. A total of 28 first-order, 13 second-order, and 6 third-order stream sites were sampled (Table 1, Figure 5). The sampling site(s) along each stream were randomly selected, and independent if multiple sites were in the same stream. This allows inferences to be made for all of the first to third-order streams of the entire upper Cumberland River watershed in the three-county study area.

Fish Sampling Protocol

Forty-seven sites were surveyed by three trained and experienced fish-sampling crews. Sampling was conducted from 1 August to 13 September, 2015. Fish sampling was conducted using a backpack electrofisher in a plot-wise manner from the bottom of the reach to the top. Twelve, 2-m x 5-m plots were established at each site with a 5 m buffer zone between each plot to allow for 12 independent samples (Figure 6). Each plot was pre-designated randomly prior to arrival at the site for either the left bank, right bank, or the center of the stream. Left and right bank were positioned by facing downstream and having one side of the plot against the bank. If

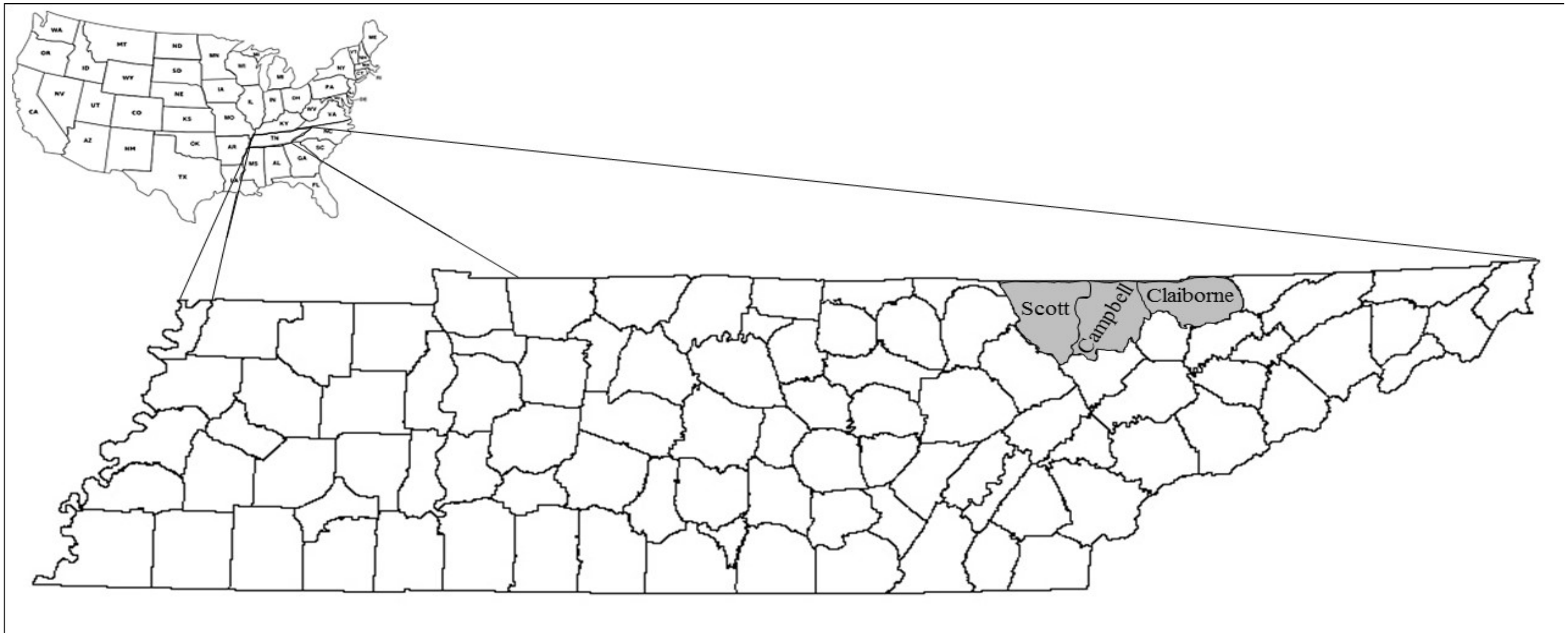

Figure 5. Expanded map showing the sampling region of this study in grey with counties of the study area labeled. USA map from URL: http://www.clker.com/cliparts/O/H/G/M/z/f/usa-map-with-state-abbreviations-md.png, Tennessee map from URL: http://www.yellowmaps.com/maps/img/US/blank-county/Tennessee_co_lines.jpg.

Table 1. List of sites sampled with GPS coordinates, stream order, and watershed area for each site. (UT= Un-named tributary)

Site #	Stream Name	Latitude	Longitude	Stream Order	Watershed Area(mi^2)	Site #	Stream Name	Latitude	Longitude	Stream Order	Watershed Area(mi^2)
1	Sugan Branch	36.5658	-83.8156	1	0.7	25	Davis Creek	36.4758	-84.0165	2	4.25
2	Mike Branch	36.5780	-84.3451	1	1.4	26	Elk Creek	36.4467	84.2952	2	6.88
3	Bear Branch	36.5205	-84.3037	1	1.25	27	Straight Creek	36.5369	-83.9004	2	3.94
4	Gum Fork	36.5639	-84.3794	1	0.75	28	Jim Branch	36.4687	-84.1451	2	7.4
5	Little Yellow Creek	36.5630	-83.7630	1	1.38	29	Capuchin Creek	36.5764	-84.2695	2	15.35
6	UT Stinking Creek	36.4821	-84.1957	1	0.52	30	Davis Creek	36.4958	-84.0654	3	14.66
7	UT Hickory Creek 2	36.4323	-84.1595	1	1.96	31	Stinking Creek	36.5062	-84.1381	3	38.45
8	Laurel Branch	36.4185	-84.2330	1	0.72	32	Stinking Creek	36.5000	-84.1316	3	39.39
9	Coontail Branch	36.4531	-84.3054	1	1.03	33	Clear Fork	36.5560	-83.9659	3	40.86
10	UT Clear Fork	36.5635	-84.0491	1	0.75	34	Tackett Creek	36.5352	-84.0036	3	33.1
11	Trammel Branch	36.5756	-84.2276	1	1.37	35	Rose Creek	36.5569	-83.9836	1	1.67
12	UT Jellico Creek	36.5391	-84.3888	1	0.72	36	Rock Creek	36.5274	-83.9364	1	1.06
13	Elk Creek	36.4389	-84.3095	1	0.92	37	Barley Branch	36.5141	-84.2346	1	1.33
14	Rock Creek	36.4813	-84.1128	1	4.64	38	Baird Creek	36.5483	-84.2631	1	1.52
15	Granny Barnes Branch	36.4937	-84.2978	1	0.56	39	UT Laurel	36.5456	-84.0941	1	1.1
16	Little Tackett Creek	36.4904	-83.9739	1	1.02	40	Childers Creek	36.5767	-84.3605	1	1.69
17	Trammel Branch	36.5449	-84.3011	1	0.93	41	UT Stinking Ck. Adams hollow	36.4726	-84.1951	1	1.05
18	Valley Creek	36.5499	-83.8608	1	0.77	42	Hogcamp Branch	36.4937	-84.0275	1	0.98
19	Hatfield Creek	36.5761	-84.2382	2	8.68	43	Tackett Creek	36.5227	-83.8301	1	4.81
20	Davis Creek	36.4627	-84.0536	2	8.41	44	Coontail Branch	36.4532	-84.2932	1	1.53
21	Little Tackett Creek	36.4993	-83.9553	2	3.87	45	Lick Fork	36.4780	-84.2919	2	5.49
22	Jellico Creek	36.5237	-84.3960	2	4.03	46	Little Elk Creek	36.5209	-84.2107	2	6
23	Burrell Creek	36.5706	-83.8081	2	3.03	47	Clear Fork	36.5715	-83.9166	3	23.07
24	Jennings Creek	36.4104	-84.2121	2	3.4						

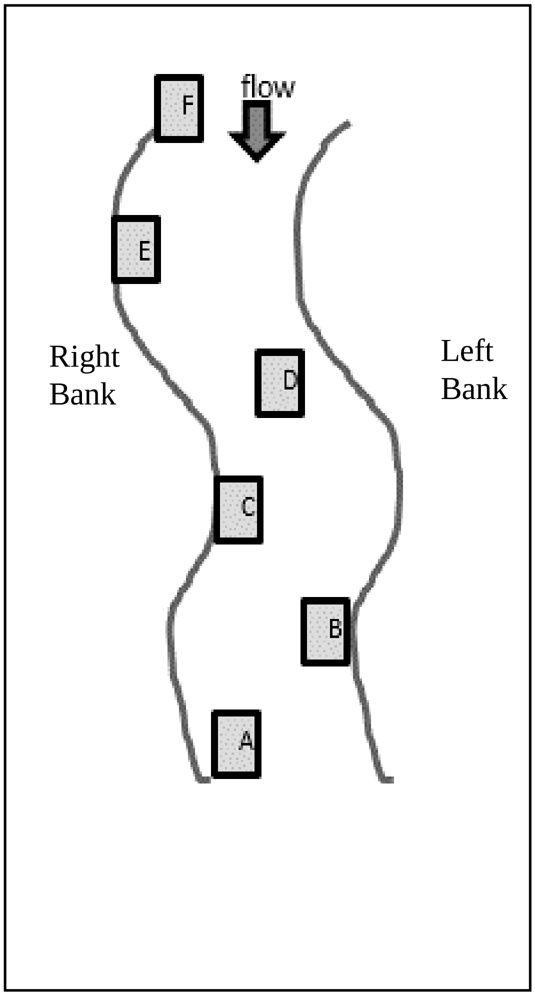

Figure 6. The layout of 5 m by 2 m sampling plots within a randomly selected stream. Plot A was placed as close to the randomly-generated geo-coordinates as possible.

the stream was less than 2 m wide, the entire width of the stream was sampled. If the stream was braided the primary channel was followed. All fishes encountered within each plot were identified and recorded on site; identifications of a few specimens were confirmed by preservation and examination in the lab at Morehead State University.

Although there was effort to have an equal sampling effort for fishes in the plots, a priority of thorough sampling meant that some plots were “shocked” longer than others. The duration of shocking conducted in each plot directly reflects its habitat heterogeneity. An open bedrock plot would not take as long to sample as a plot with high habitat heterogeneity, such as those with logs and/or root wads. Plot shocking times ranged from 4 to 158 seconds, with an average of 67.0 ± 19.1 seconds per plot (Appendix Table 1). Any presumably favorable habitat not incorporated within the 12 plots was sampled following the 12 plots. Additional sampling created a secondary data set, the supplemental data set, which combines plot-wise and additional, qualitative sampling. This provided a more thorough species survey at each site. Any additional sampling had to be near or within the reach to be included in the data set.

Water Quality Protocol

Water quality factors were measured either directly in the stream or by collecting a water samples an analyzing it the lab at Morehead State University. In the field pH, DO (mg/L), conductivity (µS/cm), and temperature (°C) were recorded at the downstream end of the reach using an YSI 550 multi-parameter probe. The probe was calibrated according to the manufacturers recommended procedure prior to fish sampling to prevent inaccurate readings (YSI Environmental 2009). The probe was recalibrated during each trip if needed or as necessary. Streams within the study region were expected to have a wide pH range because of coal mining and limestone quarries, therefore pH was calibrated using a 3 point calibration with pH 4.00, 7.00, and 10.00 standards. Conductivity was calibrated using a 718 µS/cm standard, and DO was air calibrated at each site.

At each site water quality measurements and water samples were collected before the streams were disturbed by fish sampling. Water samples were collected and stored on ice for analysis at MSU based upon recommended protocols (APHA 1998). Water samples for dissolved NH_3 and dissolved iron were acidified using concentrated HCl and stored on ice until analysis (APHA 1998). Un-acidified water samples were analyzed for TSS, alkalinity, dissolved SO_4^{2-}, dissolved PO_4^{3-}, dissolved NO_3^-, total nitrogen (TN), and total phosphorus (TP).

Total suspended solids were assessed by filtering a 500 mL sample through a weighed Watman 934AH 0.45 µm pore size glass fiber filter and oven dried (Chanin et al. 1958). The filtered water from TSS collection was used for the following analyses: alkalinity, SO_4^{2-}, PO_4^{3-}, and NO_3^-. Alkalinity was determined by titration with 0.02 N H_2SO_4 using a mixed bromocresol green/ methyl red indicator (Cooper 1941, Larson and Henely 1955, Thomas and Lynch 1960). Sulfate was determined using a turbidimetric method (Sheen et al. 1935). Phosphate was measured using the ascorbic acid method (Murphy and Riley 1962). Nitrate was determined using the sulfanimide method after cadmium reduction (Henrikson and Selmer-Olsen 1970, Nydahl 1976). The acidified samples were corrected for acidification and filtered using the same procedure used for TSS prior to NH_3 and Fe analyses. Ammoniacal N (NH_4^+ and NH_3, reported as NH_3) was measured via Nesselerization (Jenkins 1967). Dissolved Fe was determined using the colorimetric phenanthroline method (Caldwell and Adams 1946). Analyses for TN and TP were conducted on un-acidified un-filtered water samples. Total N was determined after persulfate digestion and measured as NO_3^-, and reported as N (Henrikson and Selmer-Olsen

1970, Nydahl 1976, D'Elia et al. 1977). Total P was measured as PO_4^{3-} after a modified persulfate digestion (Gales et al. 1966).

Statistical Methods

Two data sets were created from the fish collection data. The first was the plot-wise data set (quantitative) and the second was the combined (qualitative) data set. The plot-wise data represents only fishes encountered within the 12 sampling plots at each site, while the qualitative dataset incorporates any additional sampling with the plot-wise data. These two groupings allowed for comparisons between sites based upon the presence of either target species in a quantitative method, or a more traditional species survey method. This study only addresses relationships between water quality and presence data at the site level.

To determine the occupancy of both Blackside Dace and Cumberland Arrow Darters models were created using the program Presence (version 11.5) from the USGS and Proteus wildlife research consultants (Hines 2006). The simple single season analysis (1 group, Constant P) was conducted for Blackside Dace, Cumberland Arrow Darters, and the seven other most frequently-encountered species to provide occupancy estimates (Ψ) for the study region.

The Kentucky Index of Biotic Integrity (KIBI) was used as a way to estimate community health. Scores for KIBI were calculated using the procedure from the Kentucky Department of Water (Compton et al. 2003). This index incorporates species richness, total number of individuals, percent of intolerant individuals, percent of insectivorous individuals, and other metrics to produce a score representative of that community. Sampling in this study was somewhat less intensive than the KIBI protocol. However, since the method used in this study was the same at each site, KIBI results were comparable between the sites when using only the plot-wise data. Although the sampling sites are in Tennessee, they are all less than 20 km from Kentucky, and are all within the Cumberland ecoregion. The USGS program StreamStats was used to calculate watershed areas for KIBI. For watersheds less than 25.9 km^2 the headwater KIBI score is reported, and for watersheds greater than 25.9 km^2 the wadeable score is reported. Due to the defined methods of the KIBI protocol, the analysis was only conducted on first and second order streams, all third order streams were omitted.

In addition to KIBI, Shannon diversity indices (H') were calculated using the natural log to assess fish community diversity. As with KIBI, H' was calculated only from the plot-wise dataset to keep the values comparable. The plot-wise dataset had equal sampling effort at each stream and provides exact fish counts, whereas the qualitative dataset has uneven sampling efforts and was conducted as a species survey. To assess the relationship between KIBI and H' a linear regression model was used. Mann-Whitney U-tests were conducted to determine if sites with Blackside Dace and/or Cumberland Arrow Darters present had different KIBI or H' scores than sites that do not have the target species present.

To determine if any of the 13 water quality factors were significant to the presence of either target species, pairwise comparisons and multivariate analyses were used. Mann-Whitney U-tests were used to determine the statistical difference between sites with and without each target species for all water quality variables using both the plot-wise and supplemental dataset. Anderson-Darling tests indicated all water quality variables, except temperature, to be non-normally distributed, which violates an assumption of the Student's t-test (Appendix Table 2). Student's t-tests were used in place of Mann-Whitney U-tests for temperature. Box and whisker plots were used to visualize differences indicated by pair-wise comparisons to be statically significant, and for factors known to be associated with land disturbance events (SO_4^{2-} and Fe). Principal component analysis (PCA) was used to observe trends within the water quality data by incorporating eleven water quality variables. Due to sampling occurring at different times of the day temperature and DO were omitted because of known diurnal cycling. Principal component analysis scores were graphed on scatter plots with sites being labeled according to target species presence to determine if any multivariate trends exist.

To determine the relationship of conductivity to occupancy, first a correlation matrix, using Pearson Correlation analysis, was assessed to determine if significant correlations exist between conductivity and any of the water quality variables. To test the relationship between conductivity and presence proposed by prior studies, occupancy models were created using the custom model psi(Conductivity),p(.). This model was set up using the simple single season model with one covariate, conductivity. The application of conductivity as a covariate requires a threshold value; in this study the 343 µS/cm conductivity change point proposed by Hitt et al. (2016) was chosen as that threshold. This occupancy model was applied to the same nine species as the simple occupancy model described earlier. An additional analysis was conducted for the Cumberland Arrow Darter; the psi(Conductivity),p(.) model was used but the sites were split at the conductivity change point of 261 µS/cm proposed for its sister species, the Kentucky Arrow Darter (Hitt et al. 2016). A variety of different statistics and models were required to provide answers to the four study questions (Table 2).

Table 2. Statistical analyses, including data sets, purpose, and analytical methods used to examine relationships between 2015 fish presence and water quality.

Analysis Method	Data set used	Purpose
Anderson-Darling	Site water quality data	To determine if data is normally distributed and parametric
Pearson Correlation	Site water quality data	To determine if, and to what degree, the water quality variables correlate
Student's t-test	Plot-wise and supplemental fish collection data	To compare means between sites with and without target species
Mann-Whitney U-test	Plot-wise and supplemental fish collection data	To compare medians between sites with and without target species
KIBI	Plot-wise fish collection data	To assess the site quality based upon the fish community present
H'	Plot-wise fish collection data	To determine diversity of fish community present
PCA	Site water quality with both plot-wise and supplemental data overlaid	To compare all water quality variables between each sites in multi-dimensional space
Presence 11.5	Plot-wise data and water quality data	To provide occupancy estimates based upon presence/absence data

Chapter 3: Results

Water Quality

Among the 47 sites sampled a wide range of water quality factors were found, suggesting a diverse array of habitats were profiled in this study (Table 3) (Appendix Table 3). Some notable sites, with extreme values, are 19 (Hatfield Creek), 27 (Straight Creek), 32 (Stinking Creek), 41 (Adams Hollow), and 45 (Lick Fork). At site 19 TP was 1.142 mg/L-P, the highest of the sampled streams. Site 27 had a SO_4^{2-} of 390 mg/L and a NO_3^- of 0.34 mg/L, the highest of these variables. Site 32 was the warmest stream sampled with a water temperature of 25.37°C. Site 41, a stream that was bright orange in color the day it was sampled, had a conductivity of 1590 µS/cm, PO_4^{3-} of 0.819 mg/L, Fe of 12.290 mg/L, and NH_3 of 1.717 mg/L.

Conductivity was significantly correlated ($p<0.050$) with alkalinity (r= 0.528), NH_3 (r= 0.491), NO_3^- (r= 0.447), SO_4^{2-} (r= 0.896), Fe (r= 0.549), and PO_4^{3-} (r= 0.411) (Table 4). Alkalinity was significantly correlated ($p<0.050$) with NO_3^- (r= 0.349), SO_4^{2-} (r= 0.557), and pH (r= 0.584). Significant correlations ($p<0.050$) were found between pH and NH_3 (r= -0.377) and Fe (r= -0.490). Iron correlated with PO_4^{3-} ($p<0.050$, r= 0.749), while SO_4^{2-} was significantly correlated with NH_3 (r= 0.374) and NO_3^- (r= 0.478). No variable had a correlation greater than 0.900, therefore it is possible to use statistic tests without violating the no autocorrelation assumptions of PCA and other tests.

Estimated Occupancy of Blackside Dace, Cumberland Arrow Darters, and seven other commonly occurring fishes in Northeastern Tennessee

Blackside Dace were collected at six sites during plot-wise sampling, and at four additional sites during supplemental sampling (Figure 7, Table 5). Cumberland Arrow Darters were found at sixteen sites during plot-wise sampling, and at an additional two sites during supplemental sampling (Figure 8, Table 6). Presence software from USGS was used to provide

Table 3. Ranges for 13 water quality measurements from 47 sites within Scott, Claiborne, and Campbell counties in northeast Tennessee during the summer of 2015.

	Range	Average	Median	1st Quartile	2nd Quartile	3rd Quartile
Temp. (°C)	15.40 - 25.37	19.52	19.60	18.03	19.6	20.755
DO (mg/L)	2.54 - 12.82	7.68	7.45	6.89	7.45	8.665
Conductivity (µS/cm)	21.0 - 1590.0	341.9	249.0	137.1	249.0	460.5
pH	3.7 - 9.0	7.1	7.4	6.4	7.4	7.6
TSS (mg/L)	0.040 - 16.000	1.965	0.640	0.220	0.640	1.794
Alkalinity (mg/L $CaCO_3$)	0.0 - 213.0	55.8	42.0	13.3	42.0	90.3
NH_3 (mg/L)	0.011 - 1.717	0.256	0.189	0.114	0.189	0.343
NO_3^- (mg/L)	0.01 - 0.34	0.06	0.03	0.01	0.03	0.09
SO_4^{2-} (mg/L)	1.33 - 390.00	106.68	59.50	24.80	59.50	165.00
Fe (mg/L)	0.003 - 12.290	0.569	0.143	0.092	0.143	0.265
PO_4^{3-} (mg/L)	0.028 - 0.819	0.139	0.108	0.071	0.108	0.145
TN - N (mg/L)	0.228 - 38.887	2.972	1.205	0.745	1.205	1.693
TP-P (mg/L)	0.096 - 1.142	0.246	0.256	0.165	0.256	0.274

Table 4. Pearson correlation matrix of the 13 water quality variables measured from 47 sites within Scott, Campbell, and Claiborne counties in northeast Tennessee during the summer of 2015.(*=p<0.05)

	Temp. (°C)	D.O. (mg/L)	Conductivity (µS/cm)	pH	TSS (mg/L)	Alkalinity (mg/mL $CaCO_3$)	NH_3 (mg/L)	NO_3^- (mg/L)	SO_4^{2-} (mg/L)	Fe (mg/L)	PO_4^{3-} (mg/L)	TN-N (mg/L)	TP-P (mg/L)
Temp. (°C)	1.000												
D.O. (mg/L)	-0.228	1.000											
Conductivity (µS/cm)	-0.054	-0.058	1.000										
pH	-0.016	0.172	0.133	1.000									
TSS (mg/L)	0.109	-0.169	-0.162	-0.316	1.000								
Alkalinity (mg/mL CaCO3)	-0.223	-0.084	0.528*	0.584*	-0.089	1.000							
NH_3 (mg/L)	-0.027	-0.224	0.491*	-0.377*	-0.098	-0.183	1.000						
NO_3^- (mg/L)	-0.145	0.143	0.447*	0.136	-0.249	0.349*	-0.041	1.000					
SO_4^{2-} (mg/L)	-0.097	0.005	0.896*	0.213	-0.209	0.557*	0.374*	0.478*	1.000				
Fe (mg/L)	-0.082	-0.157	0.547*	-0.490*	0.036	-0.167	0.850*	0.112	0.324	1.000			
PO_4^{3-} (mg/L)	0.009	0.072	0.411*	-0.407*	-0.105	-0.256	0.639*	0.155	0.258	0.749*	1.000		
TN-N (mg/L)	-0.075	0.081	0.015	0.064	-0.103	0.007	0.113	-0.165	0.146	0.020	-0.060	1.000	
TP-P (mg/L)	0.091	-0.041	0.026	0.091	-0.079	-0.047	-0.091	0.015	-0.036	0.045	-0.031	-0.005	1.000

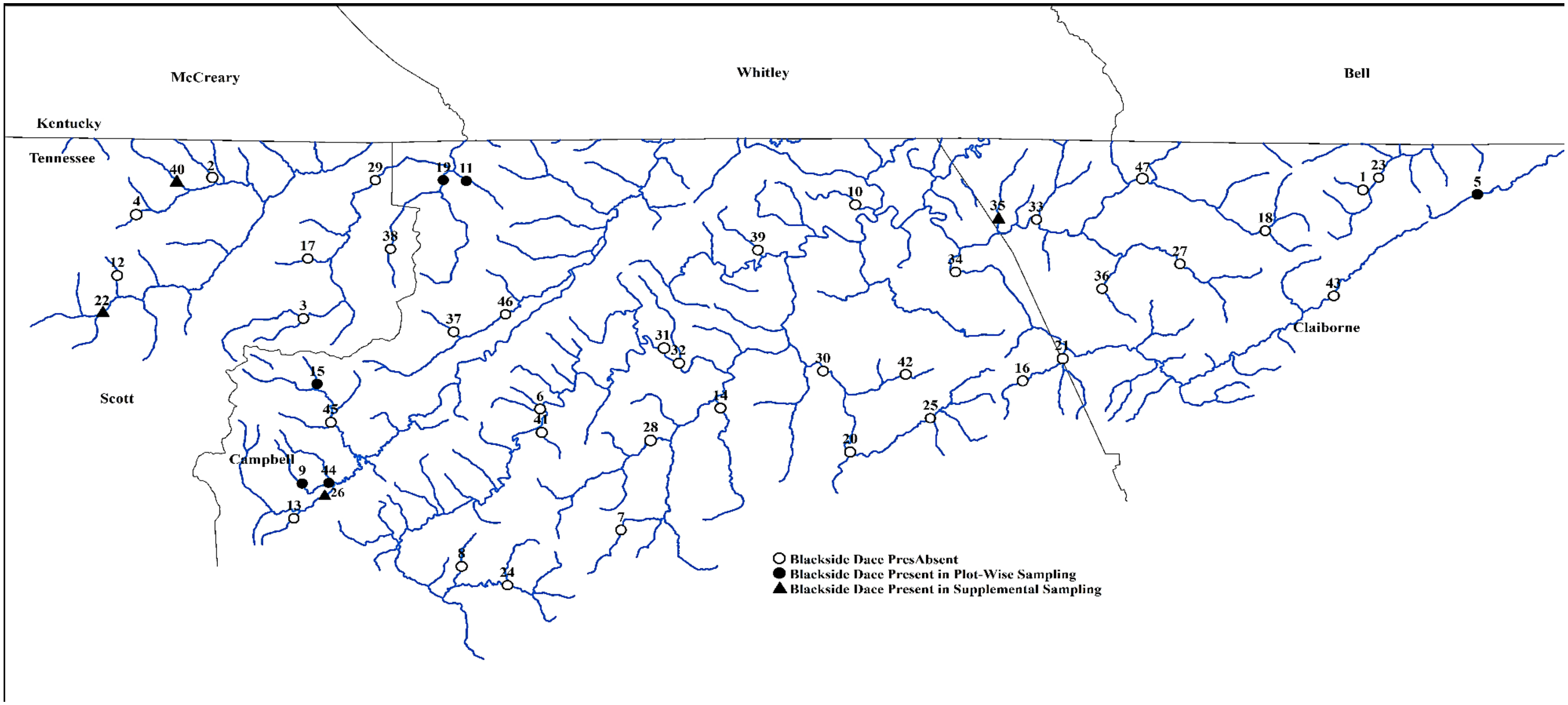

Figure 7. Distribution map of Blackside Dace detection from 47 sites within Scott, Campbell, and Claiborne counties in northeast Tennessee summer 2015.

Table 5. List of sites where Blackside Dace were detected within Scott, Campbell, and Claiborne counties in Tennessee during 2015.

Site #	Stream Name	Latitude	Longitude	Stream Order	Watershed Area(km^2)	Blackside Dace Presence
5	Little Yellow Creek	36.5630	-83.7630	1	3.57	In Plots
9	Coontail Branch	36.4531	-84.3054	1	2.67	In Plots
11	Trammel Branch	36.5756	-84.2276	1	3.55	In Plots
19	Hatfield Creek	36.5761	-84.2382	2	22.48	In Plots
22	Jellico Creek	36.5237	-84.3960	2	10.44	Supplemental
26	Elk Creek	36.4467	84.2952	2	17.82	Supplemental
35	Rose Creek	36.5569	-83.9836	1	4.33	Supplemental
40	Childers Creek	36.5767	-84.3605	1	4.38	Supplemental
44	Coontail Branch	36.4532	-84.2932	1	3.96	In Plots
45	Lick Fork	36.4780	-84.2919	2	14.22	In Plots

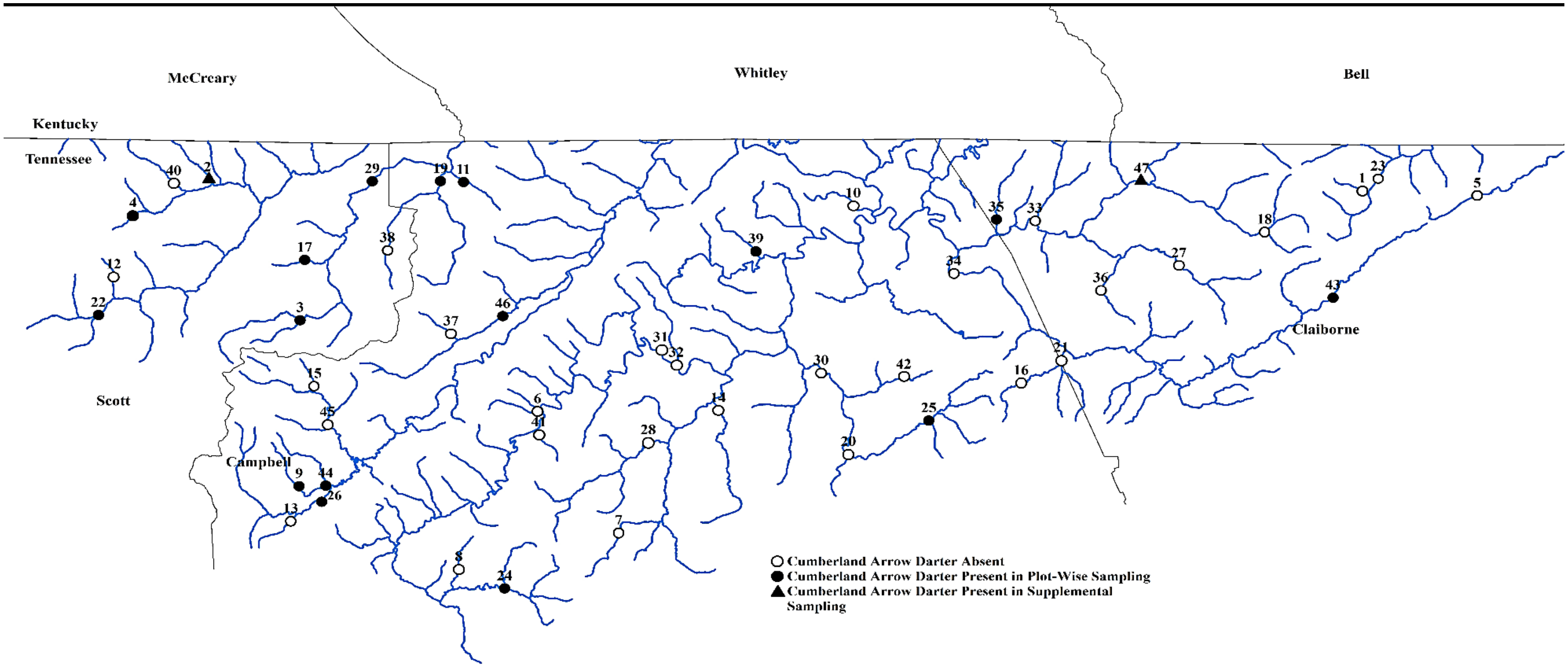

Figure 8. Distribution map of Cumberland Arrow Darter presence within Scott, Campbell, and Claiborne counties in northeast Tennessee summer 2015.

Table 6. List of sites where Cumberland Arrow Darters were detected in Scott, Claiborne, and Campbell counties in Tennessee during 2015.

Site #	Stream Name	Latitude	Longitude	Stream Order	Watershed Area(km^2)	Cumberland Arrow Darter Presence
2	Mike Branch	36.5780	-84.3451	1	3.63	Supplemental
3	Bear Branch	36.5205	-84.3037	1	3.24	In Plots
4	Gum Fork	36.5639	-84.3794	1	1.94	In Plots
9	Coontail Branch	36.4531	-84.3054	1	2.67	In Plots
11	Trammel Branch	36.5756	-84.2276	1	3.55	In Plots
17	Trammel Branch	36.5449	-84.3011	1	2.41	In Plots
19	Hatfield Creek	36.5761	-84.2382	2	22.48	In Plots
22	Jellico Creek	36.5237	-84.3960	2	10.44	In Plots
24	Jennings Creek	36.4104	-84.2121	2	8.81	In Plots
25	Davis Creek	36.4758	-84.0165	2	11.01	In Plots
26	Elk Creek	36.4467	84.2952	2	17.82	In Plots
29	Capuchin Creek	36.5764	-84.2695	2	39.76	In Plots
35	Rose Creek	36.5569	-83.9836	1	4.33	In Plots
39	UT Laurel	36.5456	-84.0941	1	2.85	In Plots
43	Tackett Creek	36.5227	-83.8301	1	12.46	In Plots
44	Coontail Branch	36.4532	-84.2932	1	3.96	In Plots
46	Little Elk Creek	36.5209	-84.2107	2	15.54	In Plots
47	Clear Fork	36.5715	-83.9166	3	59.75	Supplemental

occupancy estimations for nine species found within the study region. The twelve sampling plots served as the repeated sampling events required for occupancy modeling. Blackside Dace were found at 6 of the 47 sites, which results in a naïve occupancy of 0.1277. For Blackside Dace a detection probability (p) of 0.1390 was calculated by Presence to provide the estimated occupancy of 0.1531 ± 0.0617 (estimation ± one standard deviation) using the simple single season model (Figure 9). Cumberland Arrow Darters were found at 16 of the 47 sites, providing a naïve occupancy of 0.3404. The simple single season model estimated Cumberland Arrow Darters to have an occupancy of 0.3836 ± 0.0807 within the region, based on a detection probability of 0.1664 (Figure 9). The application of the simple single season model to the seven other most encountered species within the region shows the Creek Chub has the highest estimated occupancy (Figure 9).

Richness, Diversity, and Community Health's Relationship to the Presence of Blackside Dace and Cumberland Arrow Darters

During plot-wise sampling 2,808 fishes were collected, representing 4 families, 16 genera, and 27 species. The 4 families detected were Cyprinidae (9 species), Centrarchidae (9 species), Catostomidae (3 species), and Percidae (6 species) (Appendix Table 4). No fishes were detected at sites 1 (Sugan Branch), 12 (UT Jellico Creek), and 41 (Adams Hollow) during either plot-wise or supplemental sampling. No aquatic life was observed while sampling site 41, which had pH<4, the highest conductivity, NH_3, Fe, and PO_4^{3-}, and the second highest SO_4^{2-}. Supplemental sampling detected two additional species within the study region, Rainbow Trout (*Oncorhynchus mykiss*) and Yellow Bullhead (*Ameiurus natalis*), bringing the total number of species detected up to 29, representing 18 genera..

The most species-rich site was site 46 (Little Elk Creek) with 15 species present (Table 7); of the two target species only Cumberland Arrow Darters were present. Site 46 also had the

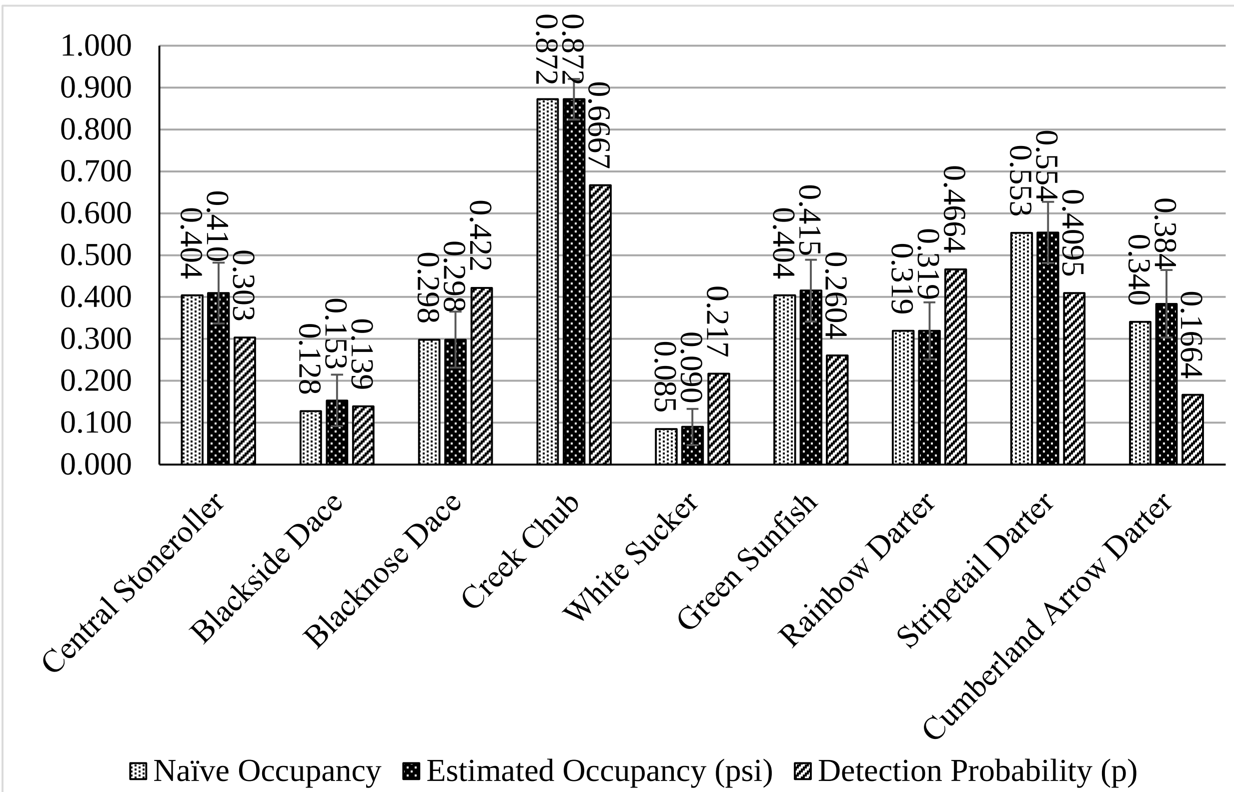

Figure 9. Naïve occupancy, estimated occupancy, and detection probability for Blackside Dace and Cumberland Arrow Darters within Scott, Campbell, and Claiborne counties from Tennessee using a simple single season model from data collected summer 2015.

Table 7. Species richness, Shannon diversity scores (H'), total number of individuals, and Kentucky index of biotic integrity (KIBI) scores for 47 sites for the plot-wise dataset from Scott, Campbell, and Claiborne counties in northeast Tennessee during summer 2015.

Site #	Species Richness	H'	KIBI	TNI	Site #	Species Richness	H'	KIBI	TNI	Site #	Species Richness	H'	KIBI	TNI
1	0	0.000	0	0	17	2	0.234	31	16	33	5	1.609	10	5
2	1	0.000	24	7	18	3	0.899	47	65	34	5	1.365	11	19
3	3	0.420	56	54	19	10	1.759	61	120	35	5	0.998	58	19
4	4	0.571	58	72	20	7	1.493	19	42	36	3	0.511	28	43
5	5	0.865	57	75	21	3	0.891	20	49	37	5	0.853	29	41
6	1	0.000	32	31	22	5	1.102	27	46	38	1	0.000	24	31
7	1	0.000	40	93	23	3	0.703	20	16	39	3	0.558	48	86
8	1	0.000	50	53	24	5	1.387	25	32	40	5	0.899	51	162
9	4	0.368	38	49	25	8	1.855	50	106	41	0	0.000	0	0
10	2	0.128	51	107	26	13	2.128	63	120	42	3	0.711	28	41
11	3	0.656	32	28	27	1	0.000	17	14	43	6	1.309	37	67
12	0	0.000	0	0	28	5	1.410	20	49	44	10	1.357	59	96
13	8	1.162	56	148	29	9	1.638	43	81	45	9	1.811	45	96
14	3	0.468	17	30	30	4	0.886	15	11	46	15	2.194	42	237
15	3	0.381	34	40	31	7	1.676	16	39	47	10	1.614	31	120
16	2	0.606	40	51	32	9	1.837	16	28					

most individuals present within the plots, 234 individuals, and the highest H’ score of 2.19 (Appendix Table 5, Table 7). Site 26 (Elk Creek) had the highest KIBI score of 63 (Table 7). Elk Creek had Blackside Dace present in supplemental sampling only, while Cumberland Arrow Darters were present in both plot-wise and supplemental sampling. Blackside Dace streams had KIBI scores that ranged from 32-61, which qualifies them as fair to excellent streams, while Cumberland Arrow Darter streams ranged from 27-63, which are classified as poor to excellent streams.

Sites with Blackside Dace present did not have statistically different species richness’, TNIs, or H’s, but all three factors were higher when Blackside Dace were present (Figure 10). Mann-Whitney U-tests indicated that sites with Blackside Dace had KIBI scores that were statistically different, and higher, than sites without ($p<0.050$). Sites with Cumberland Arrow Darters had statistically different, and higher, species richness’, TNIs, and KIBI scores compared to those without their presence ($p<0.050$) (Figure 10). As with Blackside Dace, sites with Cumberland Arrow Darters present had higher H’s, but they are not statistically different than sites without their presence (Figure 10). The calculation for H’ results in a score of 0 when only one species is present, while KIBI still provides a non-zero score. A comparison of KIBI and H’ via a linear regression model shows a positive, but nonsignificant relationship ($R^2= 0.0524$, $p=0.122$) between these variables (Figure 11).

Relationship of 13 Water Quality Variables and Blackside Dace and Cumberland Arrow Darter Presence

Mann-Whitney U-tests were used to provide a simple comparison of sites with and without Blackside Dace and Cumberland Arrow Darters for thirteen water quality factors. The plot-wise data set showed significant differences in groups, based upon a 95% confidence interval, for Blackside Dace, TN and KIBI (Table 8). For Cumberland Arrow Darters

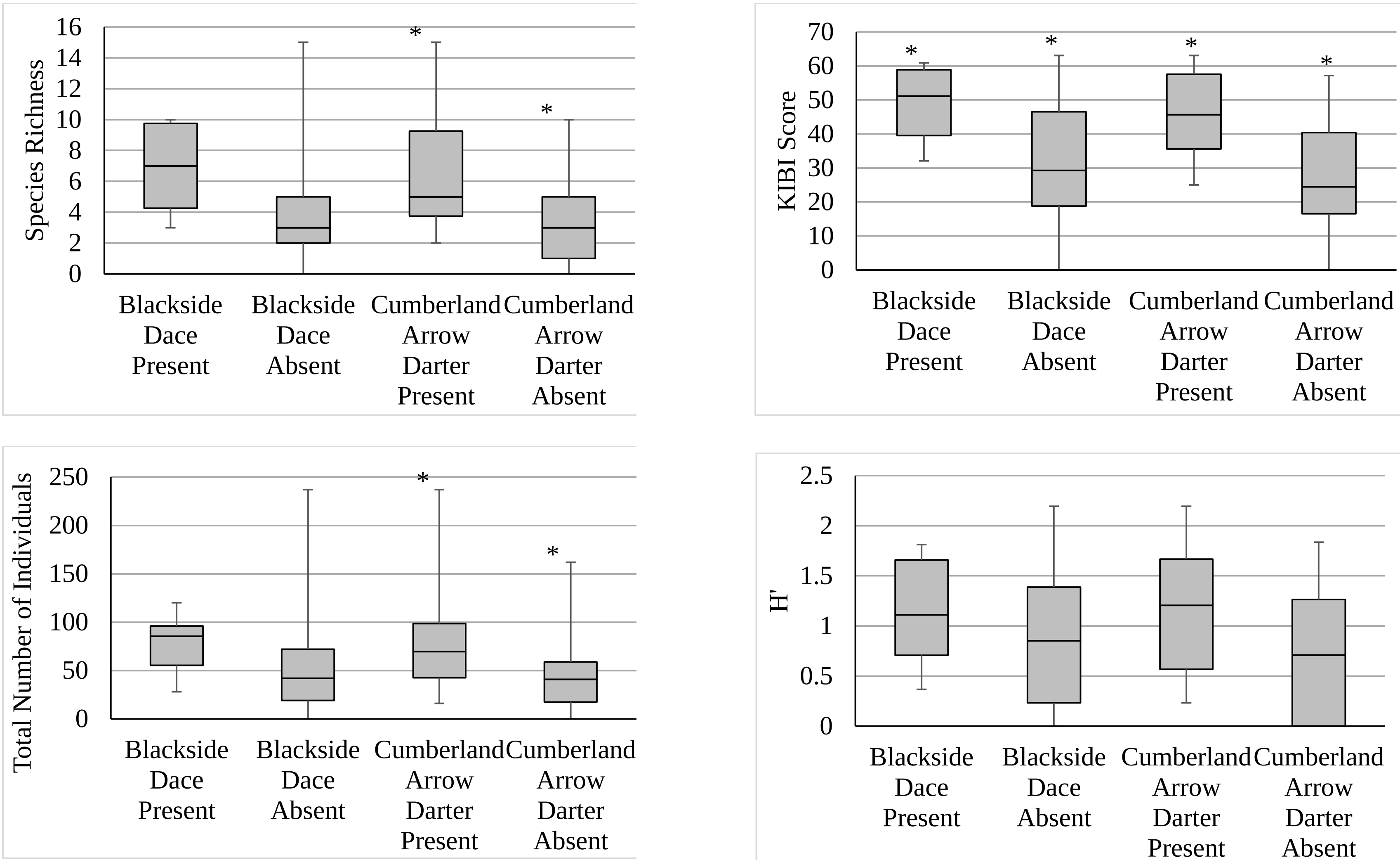

Figure 10. Box and whisker plots of species richness, total number of individuals, Kentucky index of biotic integrity (KIBI) scores, and Shannon Diversity (H') from the plot-wise sampling dataset within Scott, Campbell, and Claiborne counties in northeast Tennessee. Asterisk indicates statistical difference based upon Mann-Whitney U-test ($p<0.050$). Whiskers indicate minimum and maximum values, the gray boxes represent the first and third quartile, and the horizontal line within the boxes represents the median.

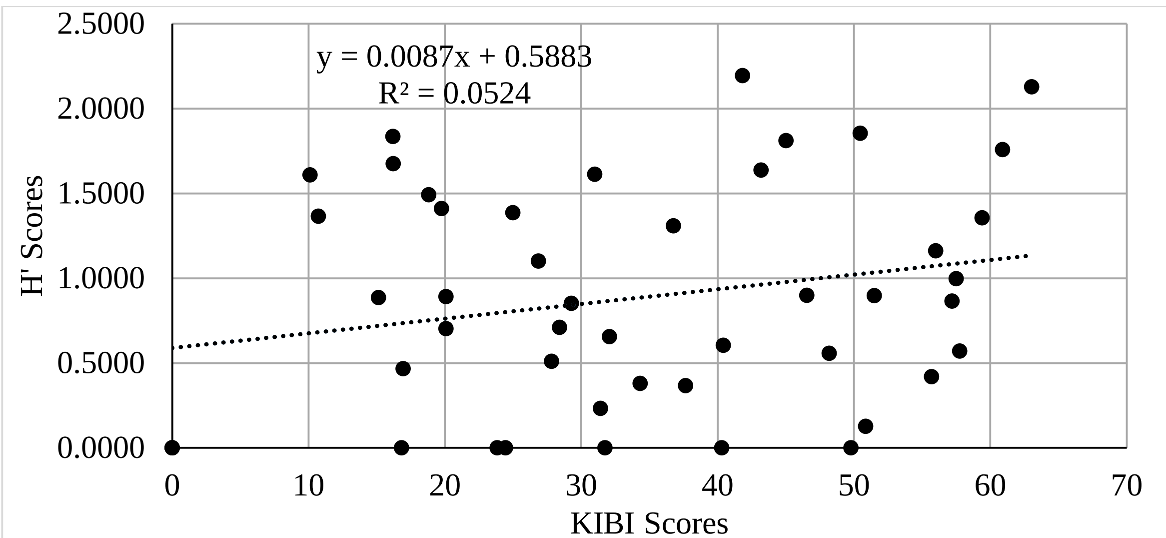

Figure 11. Linear regression model of Shannon diversity (H’) vs. Kentucky index of biotic integrity (KIBI) from plot-wise sampling of 47 sites within Scott, Claiborne, and Campbell counties in northeastern Tennessee.

Table 8. Results of Mann-Whitney U-tests using the plot-wise dataset showing medians with and without Blackside Dace (BSD) and Cumberland Arrow Darters (CAD). Water quality data was collected from 47 sites within Scott, Campbell, and Clairborne counties in Tennessee summer 2015.([1]reported as mean ± st. dev. because Student's t-test was used on normally distributed data, *indicates p<0.050)

Variable	Median with BSD present (n=6)	Median with BSD absent (n=41)	p-value	Median with CAD present (n=16)	Median with CAD absent (n=31)	p-value
Temperature (°C)[1]	19.39 ± 2.62	19.54 ± 2.21	0.9	19.4 ± 1.87	19.58 ± 2.43	0.783
D.O. (mg/L)	9.09	7.37	0.088	8.3	7.26	0.2169
Conductivity (µS/cm)	179.1	288	0.286	320*	155.6*	0.010
pH	7.55	7.4	0.355	7.39	7.45	0.400
TDS (mg/L)	0.35	0.68	0.186	0.5	0.698	0.669
Alkalinity (mg/L $CaCO_3$)	25.8	43.6	0.621	29.5	47.5	0.185
NH_3 (mg/L)	0.2545	0.186	0.599	0.174	0.207	0.884
NO_3^- (mg/L)	0.03	0.03	0.708	0.045	0.02	0.927
SO_4^{2-} (mg/L)	30	61.4	0.442	39.6	64.6	0.095
Fe (mg/L)	0.145	0.143	0.364	0.1595	0.142	0.866
PO_4^{3-} (mg/L)	0.0975	0.11	0.355	0.0865	0.11	0.248
TN (mg/L)	8.44*	1.03*	0.012	1.32	0.975	0.582
TP-P (mg/L)	0.2164	0.2564	0.798	0.1878	0.2564	0.493
KIBI	51.12*	29.29*	0.018	45.69*	24.45*	0.001
H'	1.1109	0.8532	0.345	1.2056	0.7106	0.510

conductivity and KIBI were shown to be stastically significant between sites with and without them from the plot-wise data set. Box and whisker plots of statistically significant ($p<0.050$) variables from the Mann-Whitney test and variables known to be associated with land disturbance show a trend, although not statistically significant (Figure 12). Conductivity was not stastically significant ($p<0.050$) for Blackside Dace, but the quartiles and median show sites without their presence having elevated conductivity (Figure 12). Like conductivity, SO_4^{2-} showed a non-statistically significant trend of sites with Blackside Dace present having lower SO_4^{2-} concentrations (Figure 12).

Within the supplemental dataset only conductivity was significantly different between sites with and without Blackside Dace ($p=0.050$) (Table 9). For Cumberland Arrow Darters, DO and conductivity were significantly different between sites with and without the species present ($p<0.050$). Box and whisker plots from the supplemental dataset show the same trend as the plot-wise dataset (Figure 13). Conductivity was statistically different between sites with and without either target species presence ($p<0.050$), with sites harboring the target species having lower conductivity (Figure 13). Total nitrogen shows the same trend within the supplemental dataset as in the plot-wise dataset, but it is not stastistically different between sites with or without the presence of either target species (Figure 13). Dissolved oxygen was statstically different for sites with Blackside Dace present when compared to sites where the target species are not present ($p<0.050$). For both target species DO was higher when Blackside Dace and Cumberland Arrow Darters were present compared to sites where both are not (Figure 13). Again, SO_4^{2-} was not statistically significant for either target species, but sites with either target species present tended to have lower SO_4^{2-} concentrations (Figure 13).

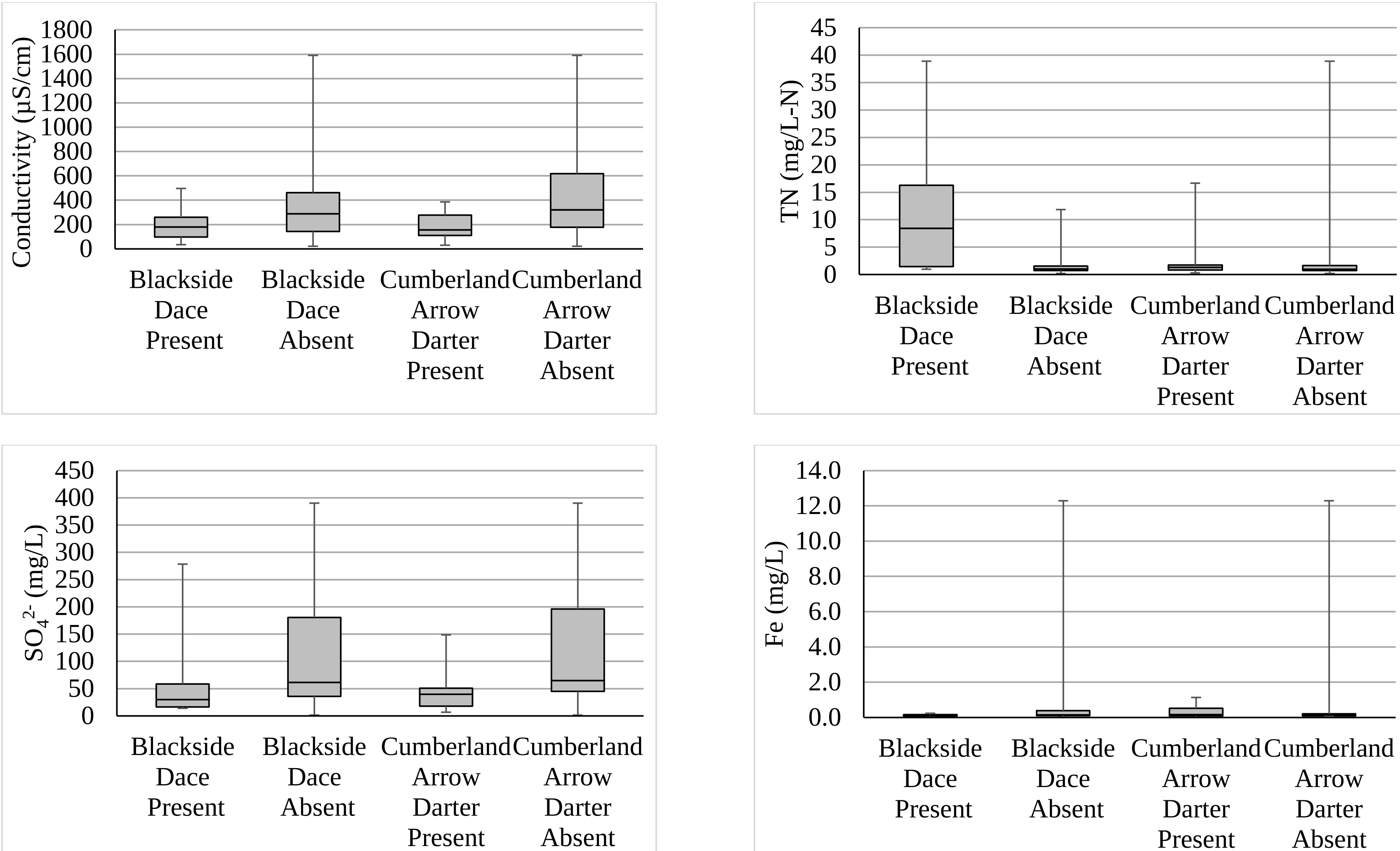

Figure 12. Box and whisker plots of four water quality factors separated by either Blackside Dace or Cumberland Arrow Darter presence. Fish presence and water quality data collected from 47 sites within Scott, Campbell, and Clairborne counties in northeast Tennessee summer 2015.

Table 9. Results of Mann-Whitney U-tests using the supplemental dataset showing medians with and without Blackside Dace (BSD) and Cumberland Arrow Darters (CAD). Water quality data was collected from 47 sites within Scott, Campbell, and Clairborne counties in Tennessee summer 2015. ([1]reported as mean ± st. dev. because Student's t-test was used on normally distributed data, *indicates $p<0.050$)

Variable	Median with BSD present (n=10)	Median with BSD absent (n=37)	p-value	Median with CAD present (n=18)	Median with CAD absent (n=29)	p-value
Temperature (°C)[1]	19.57 ± 2.25 *	19.50 ± 2.26 *	0.937	19.18 ± 1.93 *	19.73 ± 2.42 *	0.402
D.O. (mg/L)	8.615	7.26	0.070	8.515	7.23	0.029
Conductivity (µS/cm)	152.3	295	0.050	177.4	320	0.043
pH	7.36	7.45	0.897	7.39	7.45	0.381
TDS (mg/L)	0.57	0.64	0.805	0.64	0.64	0.965
Alkalinity (mg/L $CaCO_3$)	21.2	47	0.172	33.5	47	0.393
NH_3 (mg/L)	0.201	0.189	0.886	0.174	0.207	0.592
NO_3^- (mg/L)	0.045	0.2	0.842	0.045	0.02	1.000
SO_4^{2-} (mg/L)	39.8	63.1	0.242	40.5	64.2	0.080
Fe (mg/L)	0.158	0.142	0.649	0.145	0.143	0.939
PO_4^{3-} (mg/L)	0.1115	0.108	0.835	0.108	0.108	0.519
TN (mg/L)	1.46	0.98	0.081	1.32	0.975	0.646
TP-P (mg/L)	0.2678	0.2507	0.112	0.1735	0.2621	0.171

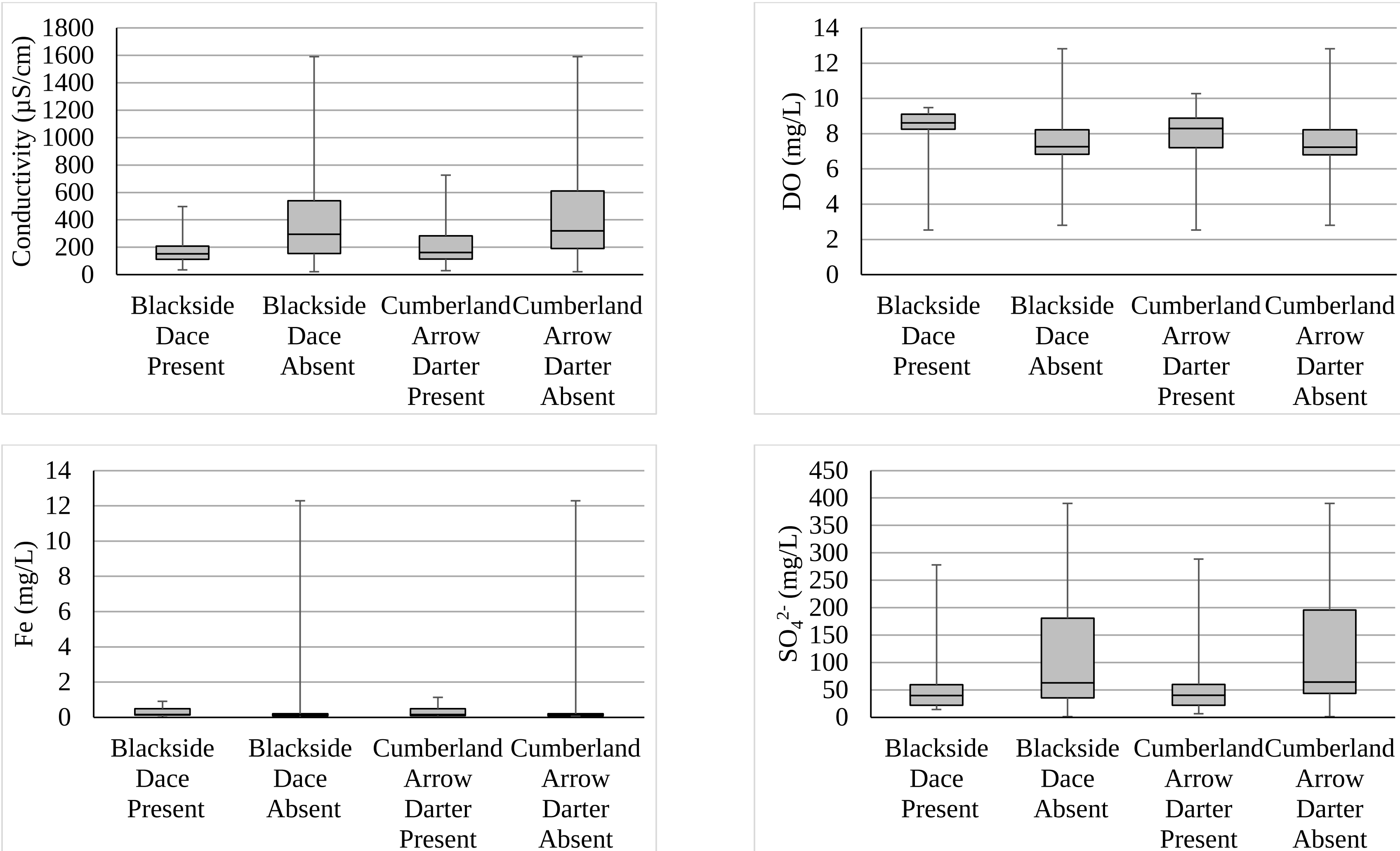

Figure 13. Box and whisker plots of four water quality factors seperated by either Blackside Dace or Cumberland Arrow Darter presence. Fish presence and water quality data collected from 47 sites within Scott, Campbell, and Clairborne counties in northeast Tennessee summer 2015.

Comparisons of means supports trends previoulsy identified by Mann-Whitney U-tests. Streams containing Blackside Dace had an average conductivity of 183 ± 130 µS/cm, as compared to 385 ± 335 in streams without them (Table 10). Streams with Cumberland Arrow Darters present had an average conductivity of 210 ± 163 µS/cm, while streams without Cumberland Arrow Darters averaged 424 ± 356 µS/cm. Alkalinity was also almost twice as high on average for streams without Blackside Dace versus with (34.6 ± 32.1 vs. 62.4 ± 52.7 mg/L $CaCO_3$). Dissolved SO_4^{2-} was over twice as high on average when Cumberland Arrow Darters were absent (61.9 ± 68.9 vs. 134.0 ± 128.0 mg/L). The non-normal distribution of these data prevents the comparison of means using a Student's t-test.

Principal component analysis was used to assess the relationship between eleven water quality factors and the presence of both target species in a multivariate fashion. Principle components (PC) 1 and 2 were only evaluated because PC3 only accounted for 11.5% of the variation, and it did not show any trends not already explained by PC1 and PC2 (Table 11). Principle component 1 accounted for 31.1% of the variation, and PC2 accounted for 15.0% of the variation; thus a graph of PC1 vs. PC2 represents 46.1% of the variation between sites based upon 11 water quality factors. The PC1 axis is primarily based on conductivity, pH, alkalinity, and dissolved SO_4^{2-}(Table 11). The highest loadings on the PC2 axis were NH_3, Fe, and NO_3^- (Table 11).

Points on a scatter plot of scores from PC1 and PC2 are individual sites, with sites to the right of the graph having higher conductivity, SO_4^{2-}, pH, and alkalinity. Sites on the upper part of the graph have higher NO_3^- and lower NH_3 and Fe. Thus, sites with Blackside Dace present are towards the upper left, which represents streams with lower conductivities, dissolved SO_4^{2-}, dissolved Fe, alkalinity, NH_3 and higher levels of dissolved NO_3^- according to the 47 sites

Table 10. Averages of the 13 water quality variables for sites with and without Blackside Dace and Cumberland Arrow Darters from the 47 sites sampled in northeastern Tennessee.

	Blackside Dace		Cumberland Arrow Darter	
Variable	Mean with ± 1 St. Dev. (n=10)	Mean w/out ± 1 St. Dev. (n=37)	Mean with ± 1 St. Dev. (n=18)	Mean w/out ± 1 St. Dev. (n=29)
Temperature (°C)	19.57 ± 2.25	19.50 ± 2.26	19.18 ± 1.93	19.73 ± 2.42
D.O. (mg/L)	8.02 ± 2.04	7.58 ± 1.53	8.21 ± 2.02	7.34 ± 1.27
Conductivity (µS/cm)	183 ± 130	385 ± 335	210 ± 163	424 ± 356
pH	7.084 ± 0.849	7.07 ± 1.19	7.038 ± 0.778	7.1 ± 1.29
TDS (mg/L)	1.24 ± 1.64	2.16 ± 3.74	1.62 ± 2.82	2.18 ± 3.76
Alkalinity (mg/L $CaCO_3$)	34.6 ± 32.1	62.4 ± 52.7	41.3 ± 36	64.8 ± 56.1
NH_3 (mg/L)	0.224 ± 0.129	0.308 ± 0.402	0.226 ± 0.141	0.275 ± 0.323
NO_3^- (mg/L)	0.047 ± 0.0327	0.0614 ± 0.0715	0.0478 ± 0.0384	0.0648 ± 0.0772
SO_4^{2-} (mg/L)	61.2 ± 78.3	119 ± 120	61.9 ± 68.9	134 ± 128
Fe (mg/L)	0.296 ± 0.299	0.64 ± 2.03	0.296 ± 0.321	0.74 ± 2.29
PO_4^{3-} (mg/L)	0.155 ± 0.127	0.135 ± 0.133	0.1077 ± 0.0714	0.159 ± 0.154
TN (mg/L)	7.9 ± 12.5	1.64 ± 2.1	3.18 ± 4.87	2.84 ± 7.24
TP-P (mg/L)	0.331 ± 0.288	0.222 ± 0.0735	0.251 ± 0.23	0.2422 ± 0.0704

Table 11. Water quality PCA loadings. Loadings > │0.250│ are highlighted, and parentheses indicated percent of variation explained by each PC axis.

Water Chemistry Variable	PC1 (31.1%)	PC2 (15.0%)	PC3 (11.5%)
Conductivity	0.498	-0.106	0.108
pH	0.392	-0.004	-0.102
TSS	-0.199	-0.029	0.654
Alkalinity	0.454	-0.088	0.261
NH_3	-0.041	-0.653	-0.236
NO_3^-	0.23	0.302	-0.043
SO_4^{2-}	0.47	-0.192	-0.004
Fe	-0.141	-0.489	0.237
PO_4^{3-}	-0.069	0.098	-0.571
TN-N	0.029	-0.38	-0.146
TP-P	0.014	0.182	0.148

sampled (Figure 14). Site 45 (Lick Fork) was the only site above the 343 µS/cm threshold to have Blackside Dace present with a conductivity of 496.7 µS/cm. Like Blackside Dace sites, Cumberland Arrow Darter sites plotted on the upper left in the PC scatterplot (Figure 15). Two sites, 46 (Little Elk Creek) and 47 (Clear Fork), had Cumberland Arrow Darters present that were above the 343 µS/cm threshold. Site 46 had a conductivity of 386.2, while site 47 had a conductivity of 727.0 µS/cm.

Conductivity and Occupancy

The application of covariates within the Presence program provides occupancy estimations for each grouping of the covariate, in this case, high (>343 µS/cm) and low (<343 µS/cm) conductivity. Higher occupancy predictions are made for five species (Blackside Dace, Creek Chubs, White Suckers, Stripetail Darter, and Cumberland Arrow Darter) when the conductivity was below 343 µS/cm, while the other four species (Central Stoneroller, Blacknose Dace, Green Sunfish, and Rainbow Darter) had higher occupancy estimations in higher conductivity streams (Figure 16). Blackside Dace in low conductivity streams have an estimated occupancy of 0.1934±0.0832, and 0.0749±0.0732 in high conductivity streams. Cumberland Arrow Darters have an estimated occupancy of 0.5089±0.1046 in lower conductivity streams, and 0.1409±0.0934 in higher conductivity streams. The conductivity change point proposed by Hitt et al. (2016) for the Kentucky Arrow Darter of 261µS/cm was used as a threshold for the Cumberland Arrow Darter to provide comparison. Using that threshold occupancy in low conductivity streams was estimated to be 0.4695±0.1164, and 0.2940±0.1044 in high conductivity streams (Figure 17).

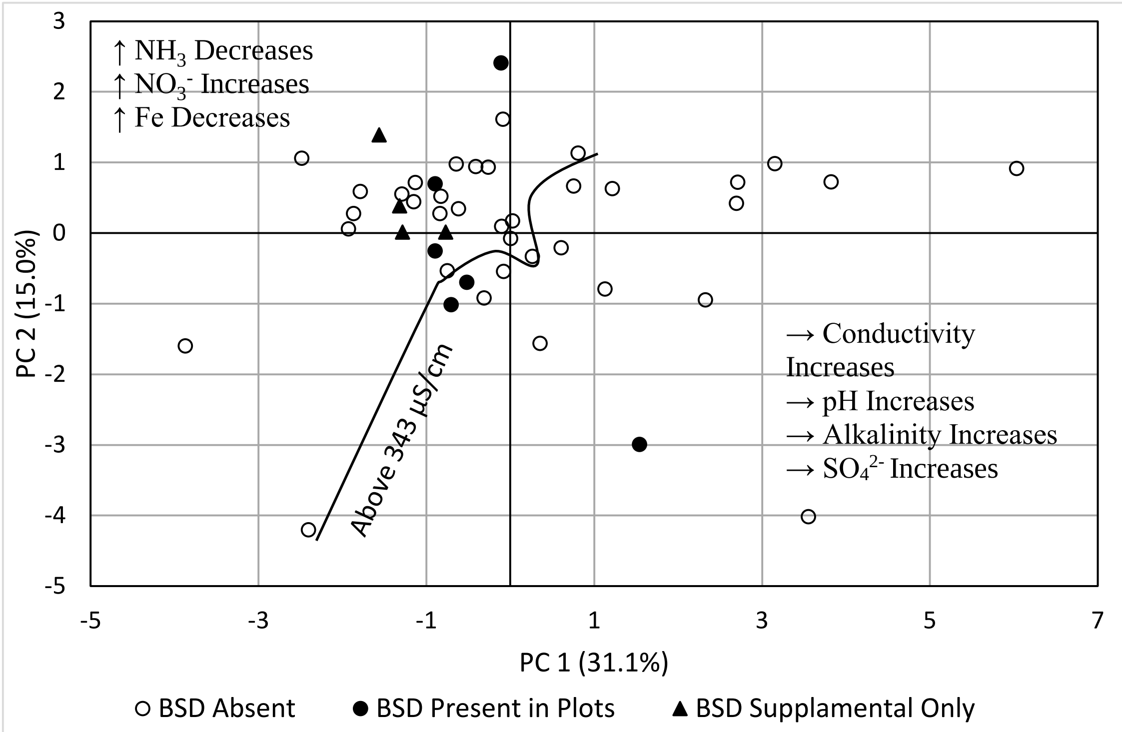

Figure 14. Principal component analysis of 11 water quality variables showing Blackside Dace (BSD) presence/absence in 46 streams (site 41 was omitted due to extreme scores).

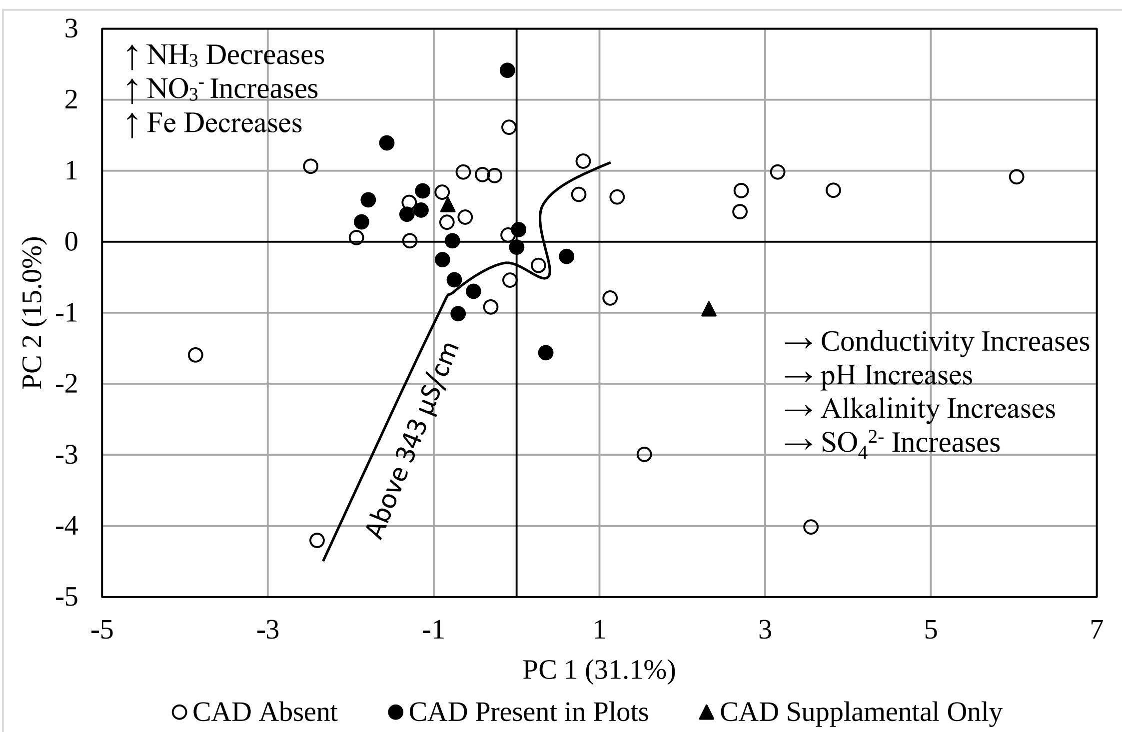

Figure 15. Principal component analysis of 11 water quality variables showing Cumberland Arrow Darter (CAD) presence/absence in 46 streams (site 41 was omitted due to extreme scores).

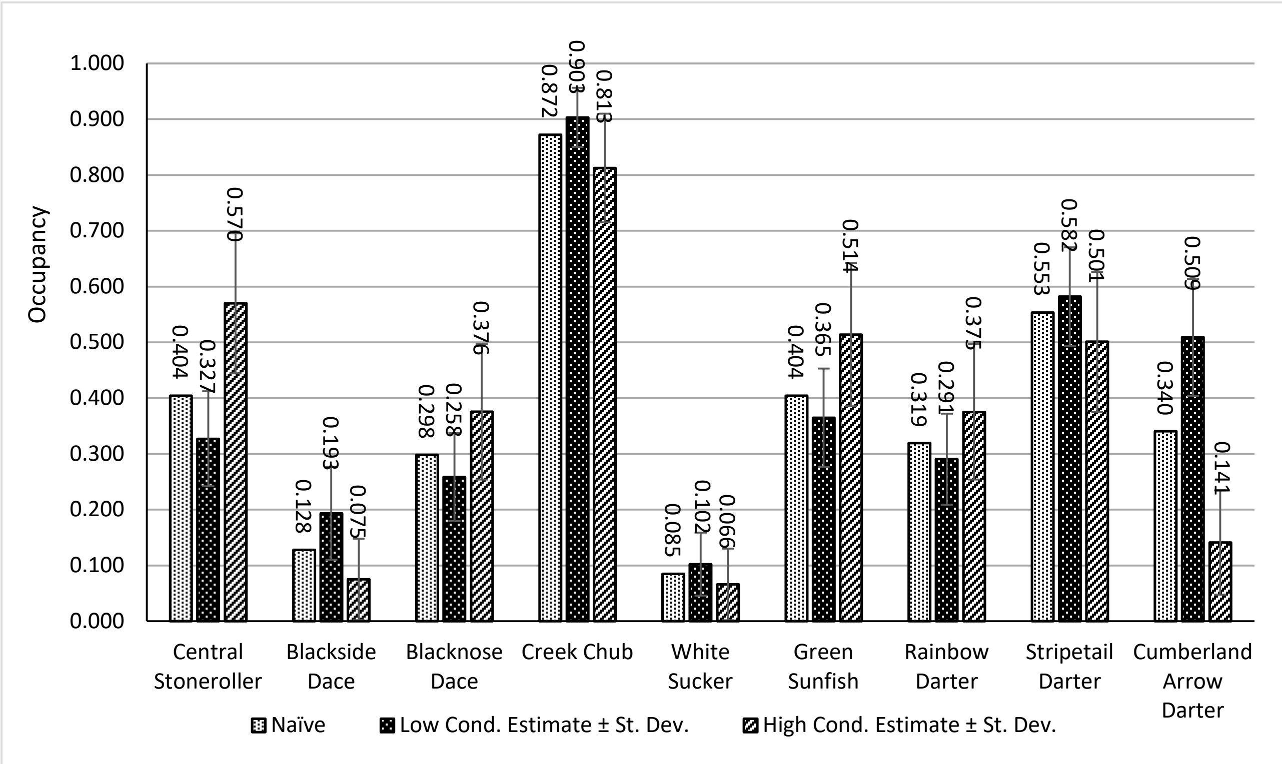

Figure 16. Occupancy model estimates for nine species using conductivity as a covariate with the threshold at 343 µS/cm. Error bars represent standard deviation.

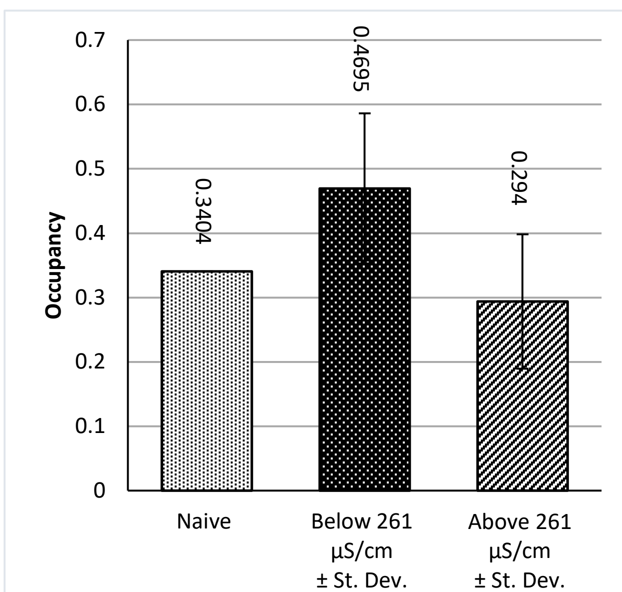

Figure 17. Occupancy model for Cumberland Arrow Darter using the 261 µS/cm threshold.

Chapter 4: Discussion

Water Quality

Water quality data collected in this study reflects extensive coal and logging activities occurring within the region, but also supports the existence of ecologically intact headwater streams. Elevated conductivity, SO_4^{2-}, Fe, and nutrient concentrations were found at many sites which is indicative of coal mining, logging, and agricultural practices (Olem 1988, Brake et al. 2001, Lindburg et al. 2011). Some of the streams sampled had small watersheds or were of extremely high gradient with waterfall barriers, precluding establishment of fish communities. These factors all contribute to the niches available which in turn determines the biotic community that can be supported through realized niches. Analysis of the 13 water quality factors discussed here is intended to clarify, increase, and bolster the growing knowledge about both Blackside Dace and Cumberland Arrow Darters within the study region.

The correlations observed among the water quality variables in this study are similar to others, such as conductivity having a significant ($p<0.050$) positive correlation with many of the ions (Wetzel 2001). Conductivity is determined by the sum of all ions in the stream, both anion and cations, therefore as ion loadings increase conductivity will increase. The significant ($p<0.050$) negative correlation observed between Fe and pH can be explained by solubility rules; lower pH causes more Fe to be in an ionized state and thus be in the water column. Significant correlations among nutrients (NH_3, NO_3^-, and PO_4^{3-}) are also not surprising, because these are common components of fertilizers, which can be carried by runoff during rain events into streams.

Many of the streams within the region had a pH above 7.0, and few streams had the low pH, often associated with coal-mining activities (Carlson 2013). The geologic strata exposed within the study region is dominated by limestone and other carbonate rocks, which results in elevated pH and alkalinity of streams when water percolates through rock exposed by coal mining and other anthropogenic activities. Many of the coal mining influenced streams in West Virginia have predominately sulfate-bearing rocks, which results in the characteristic acid mine drainage observed in Pennsylvanian coal beds.

Occupancy Modeling of Blackside Dace and Cumberland Arrow Darters in Northeastern Tennessee

Blackside Dace were found at six sites during plot-wise sampling, and at an additional four sites during supplemental sampling which reinforces just how rare these fishes are. Blackside Dace were predominately found on the western side of the study region. Based on field observations and examination of aerial photos, the western part of the study area had much less mining activity, although no analyses were conducted to determine the relationship of land disturbance events to the presence of either target species. The simple one season occupancy model predicted Blackside Dace to be present at about 15% of the sites within the region. The random sampling procedure used here allows for the conclusion made about the 47 sites to be applied all first, second, and third order streams within the study region. When the model is compared to what was found during supplemental sampling, the additional sampling supports the model. The model predicts Blackside Dace are at 7±3 sites and supplemental sampling found them at 10 sites.

When the same model was applied to Cumberland Arrow Darters it estimated them to be present at 18 ± 4 of the sites within the region. With supplemental sampling, Cumberland Arrow Darters were found at 18 sites, thus providing support for the model. The sampling method used here works well with Presence Occupancy Modeling software to provide realistic estimates about these fishes within this study region. The study design had higher detection probabilities for Cumberland Arrow Darters than Blackside Dace, but that difference was < 0.030. The plot-wise sampling method may be more suited to benthic fishes rather than pelagic, but it does work for all the fishes studied here. Even though the 12 plot sampling method provided estimates that were supported by the findings from a more thorough species survey style of sampling. This indicates the faster, less thorough, sampling method is adequate for generating occupancy models.

The occupancy models provided here show these fishes do not have high occurrence rates within the region randomly sampled. Cumberland Arrow Darters have a higher occurrence rate than Blackside Dace within the region, which supports the federal Fish and Wildlife Service's decision not to list Cumberland Arrow Darters as a protected species (USFWS 2016). These occupancy models provide a baseline for future comparison to monitor how the populations of both Blackside Dace and Cumberland Arrow Darters are changing. Resource managers could compare their results to the results of this study any number of years down the road if they use a similar random sampling method to create occupancy models. Due to the randomly selected study sites future studies would not have to survey the exact same streams, as long as they are within the same region the results would be comparable.

Richness, Diversity, and Community Health Relationship to the Presence of Blackside Dace and Cumberland Arrow Darters

Diversity scores, KIBI and H', provide numerical values that represent community structure at each site, including richness and evenness. Kentucky index of biotic integrity scores from this region above 56 are classified as excellent streams, 47-55 are good streams, 31-46 are fair streams, and below that are poor and very poor streams (Compton et al. 2003). Some streams had no fish present which results in both KIBI and H' scores being 0. Streams with only one species present have H' scores of 0 because of how the scores are calculated. Some of the streams with no fishes present, or only one species present, typically Creek Chubs, could be due to natural features (tiny stream size or high gradient). Creek Chubs can likely jump up steep riffles that other fishes may not be able to jump (personal observation). Etnier and Starnes (1993) describe how these fishes can exploit a wide range of prey items, thus allowing them to reside in streams where specialized fishes cannot reside. Creek Chubs are generalists that can exploit a wide range of niches, whereas many species have a much narrow fundamental niche.

Streams with Blackside Dace and/or Cumberland Arrow Darters present had higher diversity scores than streams without their presence, which points to these streams being of higher overall quality. Scores from KIBI also reflect that streams with Blackside Dace and Cumberland Arrow Darters present are of greater overall fish community health, however, scores for the region could be lower than expected. It can be difficult to get an accurate KIBI score for small headwater streams (Compton et al. 2003). One possible reason for lower KIBI scores is the deviation from the original sampling procedure, which likely would have resulted in a greater number of individuals being captured and better detection of both target species. Proper implementation of the KIBI procedure would have required more funding and time to sample the same number of streams. The use of seining as required by the KIBI protocol would have provided better capture rates of pelagic fishes.

Relationship of 13 Water Quality Variables and Blackside Dace and Cumberland Arrow Darter Presence

Many water quality factors have been implicated in influencing populations of certain fish species; some of these factors include temperature, heavy metal concentrations, nutrient loadings, and more recently conductivity. Factors such as dissolved metals, changes in pH, TSS, dissolved organic compounds, and dissolved ions can result in some degree of gill damage (Evans 1987, Hinton et al. 1987, Newman 2015). Tuurala and Soivio (1982) show that chloride cells on the primary lamellae of Rainbow Trout increase in number upon exposure to acid environments, which is a hypertrophic response to osmotic stress. Gills serve as the primary surface for ion and gas exchange in fish, which makes them easily susceptible to contaminants and toxins.

Often exposure results in the outer epithelium on the secondary lamellae to separate creating a fluid-filled space causing inflammation, and acting much like a blister (Skidmore and Tovelll 1972, Evans 1987, Lease et al. 2003). Fishes can clean their gills by coughing, aka gill purging, which removes or dislodges excess mucus and/or irritants that have built up. Bishop and McIntosh (1981) show that Bluegill (*Lepomis macrochirus*) have increased gill purges when exposed heavy metals. Increases in the frequency of gill purges is indicative of sublethal effects of a toxin. Dissolved metals and ions, such as Fe and SO_4^{2-}, can form salts that may be toxic to fishes and other aquatic fauna. This study did not look at gill purge rates, but it could help to explain presence or absence of intolerant endemic fishes. Trends seen in the PCA scatter plots and the Mann-Whitney U-tests show that the target species are not found as often in streams with elevated [Fe] and [SO_4^{2-}].

Principal component analysis and the Mann-Whitney U-tests both point to conductivity as the best predictor of the presence of both target species, which is consistent with the findings of other studies (Black et al. 2013, Hitt et al. 2016). The factors that created the greatest amount of separation among sites with and without the target species along PC1 and PC2 were conductivity, alkalinity, sulfate, ammonia, and iron; all factors known to increase with land disturbance (Schorr and Backer 2006). The PC1 axis, according to the PC loadings, is indicative of coal related land disturbances. More positive PC1 scores are created by sites having elevated conductivities, SO_4^{2-}, alkalinity, and pH. This type of land disturbance breaks up the parent rock and allows runoff to percolate through the disrupted parent rock strata as it travels to the streams, whereas logging and residential development do no disrupt the parent rock layers. The increased parent rock surface area allows for increased ion leaching from those previously unexposed sources.

Blackside Dace and Cumberland Arrow Darters form metapopulations connected by fishes moving among different streams, although this connectivity has been declining (Starnes and Starnes 1981, Detar and Mattingly 2013). Habitat fragmentation often occurs in the region via barriers, such as culverts, small impoundments, and beaver dams, and inhospitable mainstream habitats, created by channelization, or runoff from coal mining, logging, and agriculture that prevents the connectivity between metapopulations (Lowe 1979, Starnes and Starnes 1981, Eisenhour and Floyd 2013). Headwater streams often experience natural events (e.g., drought) leading to local extirpation of fish species. Barriers, especially anthropogenic ones, which tend to persist much longer than do natural ones (e.g., beaver dams), can prevent recolonization.

This analysis only examined water quality, but streams could vary widely in habitat, despite similarities in water quality. This could explain the absence of Blackside Dace or Cumberland Arrow Darters at many sites with low conductivities. These sites could be too high gradient for fishes, have a barrier (e.g. waterfall) preventing fish colonization, could be ephemeral, or have to small of a watershed to support Blackside Dace and/or Cumberland Arrow Darters (Eisenhour and Floyd 2013).

Conductivity and Occupancy

This study, like previous found conductivity to directly or indirectly affect fish presence (Jones 2005, Black et al. 2013, Hitt et al. 2016). How conductivity affects sensitive fishes is unclear. Hitt et al. (2016) speculates it results in changes in the benthic macroinvertebrate community that constitute winter diets for Blackside Dace. Changes in osmolarity and tonicity of stream waters in relation to the biota within can result in stress to individuals that could reduce fitness, and given time result in the extirpation of a population. Elevated conductivity may only affect critical life stages, such as early developmental stages. Conductivity measurements could act as a surrogate to indicate a variety of water quality problems. The change in ionic potential does not, by itself, need to cause damage. Increases in conductivity might occur in concert with increases in another, unmeasured factor (toxins, nutrient enrichment, etc.). Potentially conductivity might simply be a measurable surrogate for the overall disturbance within a watershed. Regardless of the mechanism of action, it is clear there is a clear negative relationship between conductivity and occupancy of Blackside Dace and Cumberland Arrow Darter.

The pair-wise comparisons between conductivity and presence show a clear negative trend even if they are not statistically significant ($p<0.050$). Inability to find statistical significance my reflect the random selection of study sites, resulting in several sites having low conductivity, but lacking other ecological requirements (e.g., watershed area, gradient, substrate) of the target species. The PCA graphs show a trend indicating both Blackside Dace and Cumberland Arrow Darters are absent or rarely occur in streams with elevated conductivities. The occupancy models support what the pair-wise comparisons and PCA scatter plots indicate – a negative relationship between conductivity and presence of intolerant species. Using the 343 µS/cm conductivity change point provided by Hitt et al. (2016) a clear difference is seen between the presence of both target species in streams with elevated conductivity vs. streams with lower conductivity. Above 343 µS/cm the occupancy rate for Blackside Dace is lower than when conductivity is below that 343 µS/cm mark. The 95% confidence interval provided by Hitt et al. (2016) includes the previous conductivity estimation for Blackside Dace by Black et al. (2013) of 240 µS/cm, and the USEPAs (2011) benchmark for harm to aquatic life of 300 µS/cm. The analysis here, both multivariate and univariate, support that a clear negative trend exists between increasing conductivity and the presence of both rare species studied here.

When the 343 μS/cm threshold from Hitt et al. (2016) is applied to seven other species it points out some species as having increased presence in streams with elevated conductivity (Figure 16). Species such as Creek Chubs, Blacknose Dace, Central Stoneroller, and the Green Sunfish are regarded as tolerant fishes by the KIBI procedure (Compton et al. 2003), and all of these fishes, except Creek Chubs, have higher predicted occupancies in streams with conductivity above 343 μS/cm. Rainbow Darters are also predicted to have higher occupancies in streams with conductivities above 343 μS/cm, but it is regarded as a more tolerant darter than the Cumberland Arrow Darter (Compton et al. 2003). Of the nine species compared in the conductivity based occupancy models Blackside Dace and Cumberland Arrow Darters showed the greatest magnitude of change between high and low conductivity sites. These two species show a different response to changes in conductivity that do the other seven species modeled, which could explain the conservation concern for both species. These models are not as counterintuitive as one might think, as conductivity increases, fishes occupying specialized niches, i.e., Blackside Dace and Cumberland Arrow Darters, begin to decline, thus creating vacant niches that can be occupied by generalist species.

Green Sunfish are known to suppress native fish populations when introduced to both streams and ponds where they will rapidly increase in numbers (Lemly 1985). These fishes are native to the region, but numerous sedimentation ponds created in association with coal extraction, might be allowing this further expansion into headwater streams. The large number of Green Sunfish found also likely reflects degradation of habitat. Overall, streams with elevated conductivities show a trend of communities shifting from intolerant endemic fishes, to tolerant generalists.

Conservation Implications

The Tennessee Board of Water Control, Oil and Gas (2013) has set standards for surface waters within the state. The temperature of surface waters are not to be affected by any activity that raises the temperature 3°C above upstream control points. The maximum level established for TSS is 500 mg/L, and should not be exceeded by any activity within the state. The also specifies that no floating materials, deposits, or color changes should be created in the surface waters of the state. In this study no streams were found that exceeded the 500 mg/L TSS limit, but two orange colored streams were observed. The Tennessee criteria set for pH is that it shall not exceed 9.0 or go below 6.0, and it may not vary by more than 1.0 unit during a 24 hour period. In this study five streams had a pH below 6.0, but none were above pH 9.0. The USEPA (2011) conductivity benchmark for biological impairment is 300 µS/cm, which was exceeded by 19 sites in this study. These standards are set to help protect aquatic biota from the extremes, however intolerant species may be harmed well before their standards are met or exceeded.

Both state and federal agencies authorize permits for mining activities that ideally limit disturbances to both aquatic and terrestrial flora and fauna. While these permits do protect against rampant disregard for the environment, they rely on monitoring to ensure regulations are not being broken during mining activities. This study, and others studies cited, support the idea that conductivity can serve as a proxy for land disturbance and also a surrogate for stream health. Many of the aforementioned studies use or relate to conductivity in some regard, and many researchers use conductivity to assess the fish community present. Models have been provided here, and by others, that support the use of conductivity as a way to monitor potential impacts to stream health (USEPA 2011, Black et al. 2013, Hitt et al. 2016). What makes conductivity attractive as a way to monitor stream health that is a relatively cheap and easy measurement to obtain. A simple, reasonably inexpensive probe is all that is required, and the result are acquired in seconds. No water samples have to be taken back to the lab for analysis. Therefore, new regulations could set standards by using conductivity values to protect streams and the biota within. New standards would need to regionally benchmarked to geology and stream order.

Consistent monitoring of stream conductivity would be recommended below all land disturbance events, not just mining activities. Regularly visiting a stream to monitor conductivity would also allow the field biologist to make observational conclusions about the stream by monitoring color and siltation. Many other factors influence the presence of both Blackside Dace and Cumberland Arrow Darters, including both biotic and abiotic factors, but conductivity could provide an easy proxy for water quality.

Conclusions

In this study base line occupancy values were established for Blackside Dace, Cumberland Arrow Darters, and for seven other commonly encountered species within Scott, Campbell, and Claiborne counties in northeastern Tennessee. Blackside Dace were predicted to inhabit 15.3% of streams within the region, while Cumberland Arrow Darters are predicted to inhabit 38.3%. This baseline could serve as a point for later comparison to determine if any of these species are increasing or decreasing within the study region, serving as a valuable tool for resource managers. This study differs from many previous studies by using an entirely new dataset collected during the summer of 2015, comprising 47 sites from three counties in northeastern Tennessee.

Of the thirteen water quality factors analyzed here, conductivity was the best indicator of the presence of both Blackside Dace and Cumberland Arrow Darters. Conductivity had a high correlation with alkalinity, NH_3, SO_4^{2-}, and Fe which are all factors known to be released by land disturbance events, such as coal mining, and to a lesser degree logging, agriculture, and residential development (Minear & Tschantz 1976, Olem 1988, Brake et al. 2001, Lindberg et al. 2011). The use of conductivity as an indicator of Blackside Dace and Cumberland Arrow Darter presence is supported in this study, using a random dataset that is independent of all studies prior. The water quality factors and trends observed here substantiate those of prior studies regarding Blackside Dace and Cumberland Arrow Darters (Lowe 1979, Starnes and Starnes 1981, Black et al. 2013, Hitt et al. 2016).

The use of occupancy models with a conductivity threshold set at 343 µS/cm showed that Blackside Dace and Cumberland Arrow Darters have lower estimated occupancies in streams above that threshold. Of the nine species that conductivity threshold occupancy models were created for, Blackside Dace and Cumberland Arrow Darters showed the greatest response to the threshold. This supports these fishes being sensitive to increases in conductivity as previously suggested before (Jones et al. 2005, Black et al. 2013, Hitt et al. 2016). Streams containing Blackside Dace and Cumberland Arrow Darters had significantly higher KIBI scores ($p<0.050$), and non-significantly higher H’ scores and species richness. This indicates that when either target species is present the overall community health is greater. A new conductivity standard of 350 µS/cm could help protect not only Blackside Dace and Cumberland Arrow Darters, but also the integrity of aquatic ecosystems in northeastern Tennessee.

Water quality factors, especially conductivity, need to be monitored to protect rare aquatic species that occur within the study region and beyond. This study provides independent unbiased support for all the previous studies that show a distinct relationship between conductivity and Blackside Dace and Cumberland Arrow Darter occurrence. Factors other than the water quality variables assessed in this study can, and likely are, influencing fishes in this region. Various other habitat factors are likely contributing to the presence/absence mystery for fishes within the region. Further research and analysis is required to uncover the complexities of both species presence and community composition in relation to water quality factors. Studies elucidating why various factors are correlated with presence or absence of certain species would help explain the interplay of many factors and help to ensure the survival of rare endemic fishes.

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Appendix

Tables

1. Shock time, in seconds, for each of the 12 plots at all 47 sites, with average ± 1 st. dev. for each site.
2. Results of Anderson-Darling test for normality for each of the 13 water quality variables, if $p<0.005$ then data is not normally distributed and parametric statistics cannot be used.
3. Results of water quality analysis for 47 sites sampled within Scott, Campbell, and Claiborne counties in northeast Tennessee during the summer of 2015.
4. List of species encountered during plot-wise sampling from 47 sites within Scott, Campbell, and Claiborne counties in northeastern Tennessee during the summer of 2015.
5. List of species encountered during supplemental sampling from 47 sites within Scott, Campbell, and Claiborne counties in northeastern Tennessee during the summer of 2015.

Table 1. Shock time, in seconds, for each of the 12 plots at all 47 sites, with average ± 1 st. dev. for each site.

	Plots sampled at each site												
Site #	1	2	3	4	5	6	7	8	9	10	11	12	Avg. ± St. Dev.
1	27	24	22	18	35	16	26	20	33	37	12	18	24.0 ± 7.8
2	71	42	53	69	68	59	83	43	49	75	68	53	61.1 ± 13.
3	64	69	63	61	76	69	87	59	96	48	56	58	67.2 ± 13.5
4	122	66	78	72	89	71	36	82	59	93	51	62	73.4 ± 22.1
5	79	59	34	78	83	83	96	58	40	35	76	62	65.3 ± 20.6
6	84	27	53	57	34	64	47	56	39	46	41	40	49.0 ± 15.2
7	69	95	68	?	60	80	90	91	60	68	52	61	72.2 ± 14.5
8	78	28	30	45	40	31	18	22	20	33	27	40	34.3 ± 16.0
9	44	62	38	48	82	42	49	45	56	47	47	57	51.4 ± 11.7
10	75	48	95	49	29	45	51	38	35	33	31	4	44.4 ± 23.1
11	64	57	57	71	63	53	87	65	76	73	61	47	64.5 ± 10.9
12	52	45	62	35	43	40	38	37	43	33	57	43	44.0 ± 8.8
13	51	65	56	47	55	55	65	85	70	53	111	66	64.9 ± 17.8
14	51	72	83	49	55	49	67	48	42	58	66	62	58.5 ± 11.8
15	43	79	44	40	78	34	71	40	57	58	52	69	55.4 ± 15.7
16	65	60	81	38	60	56	64	45	63	72	77	69	62.5 ± 12.2
17	85	58	51	73	63	77	53	60	61	83	100	81	70.4 ± 15.0
18	56	69	43	46	64	72	59	77	34	58	47	53	56.5 ± 12.7
19	80	102	84	76	78	85	76	83	88	94	86	49	81.8 ± 12.7
20	69	85	112	57	71	115	48	84	87	118	65	97	84.0 ± 23.0
21	96	56	50	109	86	117	87	60	110	92	92	77	86.0 ± 21.7
22	83	97	70	67	61	58	69	82	64	106	74	74	75.4 ± 14.4
23	72	48	61	49	50	54	67	90	41	57	52	61	58.5 ± 13.1
24	84	79	73	57	51	91	89	27	75	53	80	101	71.7 ± 20.9
25	96	92	74	83	102	108	74	72	70	62	103	123	88.3 ± 18.5
26	77	83	99	98	88	75	62	57	56	75	69	74	76.1 ± 14.2
27	53	67	57	36	75	63	47	64	59	51	49	77	58.2 ± 11.8
28	58	59	64	110	79	74	102	60	90	89	84	105	81.2 ± 18.6
29	128	87	76	92	72	117	94	104	85	93	103	80	94.3 ± 16.5
30	52	63	54	88	66	52	65	62	72	103	77	104	71.5 ± 18.2
31	129	122	124	45	93	110	78	85	81	126	97	72	96.8 ± 26.1
32	158	89	76	68	41	50	65	110	94	59	85	71	80.5 ± 31.0
33	82	61	106	107	60	59	83	66	69	52	88	62	74.6 ± 18.4
34	90	74	107	61	86	66	77	76	120	86	131	85	88.3 ± 21.1
35	45	125	79	121	75	70	79	80	91	72	77	72	82.2 ± 21.8

Table 1. Continued

	Plots sampled at each site												
Site #	1	2	3	4	5	6	7	8	9	10	11	12	Avg. ± St. Dev.
37	81	59	46	48	39	54	46	41	47	32	57	60	50.8 ± 12.6
38	74	56	59	61	59	89	76	60	59	77	73	82	68.8 ± 11.0
39	54	25	48	75	50	75	63	107	50	101	44	42	61.2 ± 24.3
40	80	71	44	89	57	80	55	58	54	97	68	67	68.3 ± 15.7
41	28	31	40	30	50	31	36	36	40	30	33	23	34.0 ± 7.0
42	103	92	94	51	56	62	86	72	83	53	51	81	73.7 ± 18.6
43	141	77	113	99	53	112	85	107	93	96	112	102	99.2 ± 21.7
44	70	88	57	69	69	86	34	73	20	82	43	55	62.2 ± 21.1
45	85	76	53	73	62	40	88	79	67	85	99	70	73.1 ± 16.2
46	118	118	75	86	61	73	92	83	92	98	80	70	87.2 ± 17.7
47	107	81	75	99	104	94	93	112	100	85	80	108	94.8 ± 12.2

Table 2. Results of Anderson-Darling test for normality for each of the 13 water quality variables. If $p<0.005$ then data is not normally distributed and parametric statistics cannot be used.

Variable	Anderson Darling Score	p-value
Temperature	0.211	0.849
DO	1.555	<0.005
Conductivity	2.05	<0.005
pH	1.73	<0.005
TDS	7.384	<0.005
Alkalinity	1.55	<0.005
NH_3	3.52	<0.005
NO_3^-	3.328	<0.005
SO_4^{2-}	3.459	<0.005
Fe	12.108	<0.005
PO_4^{3-}	4.855	<0.005
TN	10.638	<0.005
TP	5.403	<0.005

Table 3. Results of water quality analysis for 47 sites sampled within Scott, Campbell, and Claiborne counties in northeast Tennessee during the summer of 2015.

Site #	Temp (°C)	DO (mg/L)	Conductivity (µS/cm)	pH	TSS (mg/L)	Alkalinity (mg/L $CaCO_3$)	NH3 (mg/L)	NO_3^- (mg/L)	SO_4^{2-} (mg/L)	Fe (mg/L)	PO_4^{3-} (mg/L)	TN-N (mg/L)	TP-P (mg/L)	TP-PO_4^{3-} (mg/L)
1	18.8	12.82	295	5.82	1.728	54	0.039	0.16	79.8	0.126	0.162	0.23	0.16	0.51
2	20.1	7.37	155.5	6.35	0.46	0	0.249	0.13	46.4	0.193	0.028	1.38	0.16	0.49
3	18.1	8.95	110.9	7.61	0.68	7.6	0.466	0.08	45.6	0.197	0.118	1.89	0.16	0.51
4	19.1	8.66	313.3	7.46	0.2	32	0.403	0.12	148.5	0.003	0.06	1.20	0.18	0.54
5	16.6	9.14	35.2	7.89	0.94	17.6	0.188	0.06	15.4	0.242	0.133	0.98	0.23	0.72
6	18.61	7.26	206	6.42	0.2	12	0.22	0.03	9.9	0.054	0.156	0.52	0.16	0.51
7	17.67	6.68	320	8.06	5	116	0.018	0.09	43.6	0.143	0.06	3.16	0.30	0.91
8	17.58	2.8	59	6.22	1.26	25.9	0.648	0.01	6.2	2.477	0.072	11.83	0.46	1.42
9	15.8	9.48	142.2	7.62	0.76	27	0.353	0.02	40.8	0.153	0.098	15.16	0.27	0.84
10	19.62	7.38	21	4.52	0.22	4	0.12	0.02	4.9	0.08	0.123	0.98	0.27	0.82
11	19.7	8.4	80.8	7.31	0.22	24.6	0.214	0.04	14.2	0.005	0.125	16.66	0.20	0.61
12	17.6	9.35	143.3	7.18	0.16	9	0.234	0.04	59.5	0.085	0.089	1.20	0.12	0.37
13	15.4	8.9	32.1	7.58	3.8	121.6	0.108	0.02	12.6	0.082	0.161	0.23	0.28	0.86
14	20.65	6.48	398	7.4	0.34	55.4	0.333	0.01	149.5	0.127	0.222	0.80	0.25	0.77
15	19.71	6.96	692	7.81	5.8	87.6	0.069	0.01	61.4	0.08	0.127	0.75	0.27	0.84
16	19.94	6.1	41	4.24	13.2	1	0.26	0.01	7.1	1.711	0.078	0.69	0.11	0.35
17	19.6	7.45	32.3	7.37	0.22	8.4	0.294	0.06	9.1	0.045	0.137	0.57	0.16	0.51
18	17.14	7.23	441	7.3	0.698	108.4	0.142	0.08	134.25	0.118	0.087	0.63	0.31	0.94
19	21.7	9.13	273.7	7.68	0.04	10	0.011	0.05	64.6	0.028	0.063	1.72	1.14	3.50
20	23	6.63	202	6.24	16	35.4	0.186	0.01	64.6	0.288	0.098	1.89	0.26	0.80
21	20.86	6.83	460	6.18	3	14.8	0.419	0.01	195.5	0.099	0.077	0.46	0.20	0.63
22	20.1	8.63	109	7.17	1.86	10.8	0.152	0.07	38.9	0.909	0.306	1.15	0.28	0.86
23	19.36	6.92	680	7.56	1.258	113.4	0.054	0.18	327.5	0.086	0.108	0.92	0.26	0.79
24	16.42	6.88	169	6.25	4	68.6	0.052	0.01	40.3	0.386	0.139	1.32	0.28	0.86
25	21.05	6.79	132	6.21	11.4	35	0.14	0.01	35.6	0.166	0.055	2.41	0.15	0.45

Table 3. Continued

Site #	Temp (°C)	DO (mg/L)	Conductivity (µS/cm)	pH	TSS (mg/L)	Alkalinity (mg/L $CaCO_3$)	NH3 (mg/L)	NO_3^- (mg/L)	SO_4^{2-} (mg/L)	Fe (mg/L)	PO_4^{3-} (mg/L)	TN-N (mg/L)	TP-P (mg/L)	TP-PO_4^{3-} (mg/L)
26	18.6	8.2	186.2	7.41	5.4	69.6	0.161	0.01	30.3	0.571	0.044	0.57	0.30	0.91
27	17.12	7.58	1059	7.96	0.14	213	0.12	0.34	390	0.172	0.122	1.66	0.27	0.82
28	20.56	6.7	461	6.62	0.14	20	0.36	0.01	196.25	0.205	0.26	1.49	0.27	0.82
29	22.4	7.14	310	7.48	0.4	43.6	0.167	0.06	106.5	0.52	0.177	0.63	0.16	0.51
30	23.61	7.02	540	7.64	1.18	91.6	0.374	0.02	180.5	0.196	0.15	0.98	0.26	0.80
31	23.72	6.57	248	7.94	0.4	47	0.207	0.01	63.1	0.157	0.046	2.41	0.26	0.79
32	25.37	7.9	249	7.45	0.14	47.5	0.226	0.01	1.33	0.142	0.134	0.98	0.27	0.82
33	22.23	7.46	623	8.96	0.3	116	0.064	0.11	186.5	0.15	0.07	1.43	0.23	0.72
34	21.18	7.82	611	8.81	0.22	121.2	0.189	0.13	181	0.138	0.106	0.75	0.26	0.79
35	18.25	7.01	125	4.97	0.22	14.6	0.09	0.09	46.1	0.132	0.218	1.55	0.26	0.80
36	18.62	6.8	803	8.65	0.34	105	0.083	0.18	327.5	0.113	0.056	1.32	0.26	0.80
37	18.1	9.23	294.3	7.62	2	42	0.081	0.02	47.9	0.06	0.083	0.75	0.27	0.84
38	19.4	8.22	190.5	7.3	0.16	33	0.156	0.06	48.7	0.081	0.374	0.86	0.10	0.30
39	19.9	9.23	29	6.15	0.6	9	0.181	0.02	6.6	0.098	0.062	0.29	0.15	0.45
40	22.4	8.6	162.4	6.76	2.26	2.6	0.369	0.1	64.2	0.619	0.431	1.03	0.27	0.84
41	18.71	7.07	1590	3.7	1.4	0	1.717	0.13	385	12.29	0.819	3.10	0.28	0.86
42	16.68	6.9	929	8.46	0.64	183.6	0.703	0.01	378.13	1.121	0.11	1.61	0.19	0.58
43	17.96	7.55	288	7.56	0.3	99	0.06	0.05	12.3	1.137	0.067	1.32	0.23	0.72
44	22.04	2.54	216	6.54	0.38	89	0.406	0.02	19.3	0.137	0.035	1.38	0.17	0.52
45	20.5	9.05	496.7	7.49	0.32	80	0.295	0.01	278.13	0.163	0.097	38.89	0.18	0.56
46	19.7	8.67	386.2	7.6	0.96	82	0.448	0.01	120	0.525	0.075	0.92	0.11	0.35
47	16.1	10.27	727	7.94	1	112	0.216	0.01	288.75	0.128	0.131	7.06	0.14	0.44

Table 4. List of species encountered during plot-wise sampling from 47 sites within Scott, Campbell, and Claiborne counties in northeastern Tennessee during the summer of 2015.

Species\ Site Number	1	2	3	4	5	6	7	8	9	10
Campostoma anomalum										
Chrosomus cumberlandensis					11				2	
Chrosomus erythrogaster									1	
Luxilus chrysocephalus										
Lythrurus fasciolaris										
Notropis rubellus										
Pimephales notatus										
Rhinichthys obtusus					1					3
Semotilus atromaculatus		7	48	60	55	31	93	53	45	104
Catostomus commersonii					2					
Hypentelium nigricans										
Moxostoma erythrurum										
Ambloplites rupestris										
Lepomis auritus										
Lepomis cyanellus				2						
Lepomis gulosus										
Lepomis macrochirus										
Lepomis megalotis										
Micropterus dolomieu										
Micropterus punctulatus										
Micropterus salmoides										
Etheostoma baileyi										
Etheostoma blennioides										
Etheostoma caeruleum										
Etheostoma kennicotti			4	9	6					
Etheostoma sagitta			2	1					1	
Percina caprodes										

Table 4. Continued

Species\ Site Number	11	12	13	14	15	16	17	18	19	20
Campostoma anomalum			2	3	1			6	32	
Chrosomus cumberlandensis	2								5	
Chrosomus erythrogaster			7							
Luxilus chrysocephalus			1							1
Lythrurus fasciolaris										
Notropis rubellus									3	
Pimephales notatus										
Rhinichthys obtusus			48	1				21	5	
Semotilus atromaculatus	22		81	26	36	36	15	38	19	17
Catostomus commersonii										
Hypentelium nigricans									7	
Moxostoma erythrurum										
Ambloplites rupestris										1
Lepomis auritus			2							
Lepomis cyanellus						15				12
Lepomis gulosus										1
Lepomis macrochirus										
Lepomis megalotis										
Micropterus dolomieu										
Micropterus punctulatus										
Micropterus salmoides									1	
Etheostoma baileyi										
Etheostoma blennioides										
Etheostoma caeruleum			2						42	6
Etheostoma kennicotti			5		3				3	4
Etheostoma sagitta	4						1		3	
Percina caprodes										

Table 4. Continued

Species\ Site Number	21	22	23	24	25	26	27	28	29
Campostoma anomalum		4			20	13		14	35
Chrosomus cumberlandensis									
Chrosomus erythrogaster									
Luxilus chrysocephalus					2	12			
Lythrurus fasciolaris						3			
Notropis rubellus									
Pimephales notatus						6			
Rhinichthys obtusus			3		14				
Semotilus atromaculatus	31	21	12	12	17	14	14	14	11
Catostomus commersonii						3			
Hypentelium nigricans						7		1	3
Moxostoma erythrurum						1			
Ambloplites rupestris				7					1
Lepomis auritus						1			
Lepomis cyanellus	12		1		10	1			1
Lepomis gulosus									
Lepomis macrochirus				3					
Lepomis megalotis									
Micropterus dolomieu									
Micropterus punctulatus									
Micropterus salmoides									
Etheostoma baileyi									4
Etheostoma blennioides									1
Etheostoma caeruleum		1			18	22		14	
Etheostoma kennicotti	6	19		9	24	33		6	15
Etheostoma sagitta		1		1	1	3			10
Percina caprodes						2			

Table 4. Continued

Species\ Site Number	30	31	32	33	34	35	36	37	38	39
Campostoma anomalum	1		11		2			2		
Chrosomus cumberlandensis										
Chrosomus erythrogaster										
Luxilus chrysocephalus		2								
Lythrurus fasciolaris										
Notropis rubellus			2							
Pimephales notatus					1					
Rhinichthys obtusus						10	1	2		17
Semotilus atromaculatus	8		1			55	36	31	31	68
Catostomus commersonii										
Hypentelium nigricans		5	2	1						
Moxostoma erythrurum										
Ambloplites rupestris	1	8	1	1						
Lepomis auritus										
Lepomis cyanellus			1	1	3		6	5		
Lepomis gulosus										
Lepomis macrochirus										
Lepomis megalotis										
Micropterus dolomieu		2	2							
Micropterus punctulatus										
Micropterus salmoides										
Etheostoma baileyi										
Etheostoma blennioides		2								
Etheostoma caeruleum		15	5	1	9	1				
Etheostoma kennicotti	1	5	3	1	4	24		1		
Etheostoma sagitta						1				1
Percina caprodes										

Table 4. Continued

Species\ Site Number	40	41	42	43	44	45	46	47
Campostoma anomalum	3			1		28	9	18
Chrosomus cumberlandensis					5	1		
Chrosomus erythrogaster								
Luxilus chrysocephalus					1	3	47	
Lythrurus fasciolaris								
Notropis rubellus								
Pimephales notatus					1		38	2
Rhinichthys obtusus			28					7
Semotilus atromaculatus	104		12	4	61	22	38	21
Catostomus commersonii					5	3		
Hypentelium nigricans					1	2	10	9
Moxostoma erythrurum							1	1
Ambloplites rupestris								
Lepomis auritus	5			6	2		16	3
Lepomis cyanellus	3		1	37	8	11	6	
Lepomis gulosus								
Lepomis macrochirus							11	1
Lepomis megalotis							2	
Micropterus dolomieu								2
Micropterus punctulatus							1	
Micropterus salmoides							1	
Etheostoma baileyi								
Etheostoma blennioides								
Etheostoma caeruleum						18	44	56
Etheostoma kennicotti	47			6	9	8	10	
Etheostoma sagitta				13	3		3	

Table 5. List of species encountered during supplemental sampling from 47 sites within Scott, Campbell, and Claiborne counties in northeastern Tennessee during the summer of 2015. (1 indicates the species was present, nothing indicates it was absent)

Species\Location	1	2	3	4	5	6	7	8	9	10
Campostoma anomalum										
Chrosomus cumberlandensis					1				1	
Chrosomus erythrogaster									1	
Luxilus chrysocephalus										
Lythrurus fasciolaris										
Notropis rubellus										
Pimephales notatus										
Rhinichthys obtusus					1	1				1
Semotilus atromaculatus		1	1	1	1	1	1	1	1	1
Catostomus commersonii					1					
Hypentelium nigricans										
Moxostoma erythrurum										
Amerius natalis										
Ambloplites rupestris										
Lepomis auritus		1								
Lepomis cyanellus				1						
Lepomis gulosus										
Lepomis macrochirus										
Lepomis megalotis										
Micropterus dolomieu										
Micropterus punctulatus										
Micropterus salmoides										
Etheostoma baileyi										
Etheostoma blennioides										
Etheostoma caeruleum										
Etheostoma kennicotti			1	1	1					
Etheostoma sagitta		1	1	1	1				1	
Percina caprodes										
Oncorhynchus mykiss										

Table 5. Continued

Species\Location	11	12	13	14	15	16	17	18	19	20
Campostoma anomalum			1	1	1			1	1	
Chrosomus cumberlandensis	1								1	
Chrosomus erythrogaster			1							
Luxilus chrysocephalus			1						1	1
Lythrurus fasciolaris										
Notropis rubellus									1	
Pimephales notatus										
Rhinichthys obtusus			1	1				1	1	
Semotilus atromaculatus	1		1	1	1	1	1	1	1	1
Catostomus commersonii									1	
Hypentelium nigricans									1	
Moxostoma erythrurum										
Amerius natalis										
Ambloplites rupestris									1	1
Lepomis auritus			1							
Lepomis cyanellus						1				1
Lepomis gulosus										1
Lepomis macrochirus									1	
Lepomis megalotis										
Micropterus dolomieu										
Micropterus punctulatus										
Micropterus salmoides									1	
Etheostoma baileyi										
Etheostoma blennioides										
Etheostoma caeruleum			1						1	1
Etheostoma kennicotti			1		1				1	1
Etheostoma sagitta	1						1		1	
Percina caprodes										
Oncorhynchus mykiss										

Table 5. Continued

Species\Location	21	22	23	24	25	26	27	28	29	30
Campostoma anomalum	1	1			1	1		1	1	1
Chrosomus cumberlandensis		1				1				
Chrosomus erythrogaster										
Luxilus chrysocephalus					1	1				
Lythrurus fasciolaris						1				
Notropis rubellus										
Pimephales notatus					1	1				
Rhinichthys obtusus			1		1					
Semotilus atromaculatus	1	1	1	1	1	1	1	1		1
Catostomus commersonii						1				
Hypentelium nigricans						1		1		1
Moxostoma erythrurum						1				
Amerius natalis										
Ambloplites rupestris				1				1		1
Lepomis auritus						1		1		
Lepomis cyanellus	1		1		1	1				
Lepomis gulosus										
Lepomis macrochirus				1					1	
Lepomis megalotis										
Micropterus dolomieu									1	
Micropterus punctulatus										
Micropterus salmoides										
Etheostoma baileyi										
Etheostoma blennioides										
Etheostoma caeruleum		1			1	1		1		
Etheostoma kennicotti	1	1		1	1	1		1		1
Etheostoma sagitta		1		1	1	1				
Percina caprodes						1				
Oncorhynchus mykiss	1									

Table 5. Continued

Species\Location	31	32	33	34	35	36	37	38	39	40
Campostoma anomalum	1	1	1	1			1			1
Chrosomus cumberlandensis					1					1
Chrosomus erythrogaster										
Luxilus chrysocephalus	1		1							
Lythrurus fasciolaris										
Notropis rubellus	1	1	1							
Pimephales notatus	1		1	1						
Rhinichthys obtusus					1	1	1		1	
Semotilus atromaculatus		1	1		1	1	1	1	1	1
Catostomus commersonii										
Hypentelium nigricans	1	1	1	1	1					
Moxostoma erythrurum										
Amerius natalis										
Ambloplites rupestris	1	1	1							
Lepomis auritus										1
Lepomis cyanellus		1	1	1		1	1			1
Lepomis gulosus										
Lepomis macrochirus				1						
Lepomis megalotis										
Micropterus dolomieu	1	1	1							
Micropterus punctulatus										
Micropterus salmoides										
Etheostoma baileyi										
Etheostoma blennioides	1									
Etheostoma caeruleum	1	1	1	1	1					
Etheostoma kennicotti	1	1	1	1	1		1			1
Etheostoma sagitta					1				1	
Percina caprodes										
Oncorhynchus mykiss										

Table 5. Continued

Species\Location	41	42	43	44	45	46	47
Campostoma anomalum		1	1		1	1	1
Chrosomus cumberlandensis				1	1		
Chrosomus erythrogaster							
Luxilus chrysocephalus				1	1	1	1
Lythrurus fasciolaris							
Notropis rubellus							1
Pimephales notatus				1		1	1
Rhinichthys obtusus		1					1
Semotilus atromaculatus		1	1	1	1	1	1
Catostomus commersonii		1		1	1	1	
Hypentelium nigricans				1	1	1	1
Moxostoma erythrurum						1	1
Amerius natalis			1			1	
Ambloplites rupestris			1				1
Lepomis auritus		1	1	1		1	
Lepomis cyanellus		1	1	1	1	1	
Lepomis gulosus							
Lepomis macrochirus						1	1
Lepomis megalotis			1			1	
Micropterus dolomieu							1
Micropterus punctulatus						1	
Micropterus salmoides						1	
Etheostoma baileyi							
Etheostoma blennioides							
Etheostoma caeruleum					1	1	1
Etheostoma kennicotti			1	1	1	1	
Etheostoma sagitta			1	1		1	1
Percina caprodes							
Oncorhynchus mykiss							